ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editor

Elementary Analysis of the Future

ELEMENTARY analysis by micromethods is the accepted and preferred procedure. The glamour of special apparatus, small samples, and delicate balances is intriguing, especially to those outside the field. There is a rather conservative attitude among microanalysts, because in their work individual technique is so important. This attitude tends to crystallize and prevent change of method if acceptable results are obtained. Because of these things the field of elementary analysis, in so far as instrumentation is concerned, has not kept pace with the developments in spectroscopy. Here automation and high-priced gadgetry have reached a point where diminishing returns have begun to appear.

However, looking at this example of what can be done has made us believe that another look should be taken at better instrumenting of some procedures in elementary analysis. While we have felt for some years that this was feasible, the amount of research and money involved were rather discouraging. The high development costs of mass spectrometers and automatic emission spectrometers and their purchase price, running into thousands of dollars, give us courage to take a fresh look at a determination like carbon and hydrogen.

There is much handling of weighing and absorption tubes, so that even with automatic combustion it is still a rather laborious procedure. Over the years the cost of single-purpose instruments has reached a point where they are attractive for wide application. The reason for this is, of course, that an instrument—e.g., infrared—for the measurement of the concentration of one element or compound can now be built rather simply and hence at a reasonable price.

The carbon and hydrogen determination can be run in a total of 30 minutes with automatic burning equipment, but it is more likely to consume from an hour to an hour and a half. It has been demonstrated that rapid burning in 5 minutes at a high temperature, 1000° to 1500° C., and rapid sweeping of combustion products could reduce the time considerably. More research is necessary to develop a technique whereby the sample could be oxidized in 1 to 2 minutes. If this could be combined with a system whereby the carbon dioxide and water could be collected and recorded in a closed system, with the elimination of absorption tubes whose conditioning and weighing consume a large proportion of the time, then it is conceivable that the time could be cut to 5 minutes. Actually, a steel analysis for carbon can be run in this length of time, so that what we are proposing is not so impossible as present procedure practice would have us believe.

We write this therefore, not for the reason that there is not a reasonable solution to this problem, but because there is such a gap between the microanalysts and those skilled in instrument design that they are likely to continue to go their separate ways. It is to be hoped that some day a combination of interests and imaginative research will put elementary analysis on a par with other instrument methods requiring highly developed sensing and measuring equipment. To know that such things go slowly we need only look back to the history of automatic program burning furnaces. It took almost 10 years for the manufacturers and microanalysts to accept the idea that this type of furnace was practical and if built could be sold.

In time we would like to see equipment designed to measure rapidly all the combustion products from one sample. By the time this has been accomplished and accepted, we hope enough will be known to make it possible to determine quantitatively all the elements in an organic compound without destroying the sample.

Voltammetry at Constant Current

Review of Theoretical Principles

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Fundamentals of voltammetry at constant current are discussed and potentialities of the method as an analytical tool are examined. Concentrations are determined by measuring the transition time on potential-time curves. Relationships for transition-time and/or potential-time curves are discussed for reversible, irreversible, consecutive, stepwise, and kinetic processes. The influence of the capacity current in the selection of the density of electrolysis current is also considered.

THE potential of the working electrode in conventional voltammetry is controlled and the electrolysis current is the measured quantity. The opposite method, in which the current through the cell is controlled and the variations of potential of the working electrode are followed during electrolysis, has seldom been applied. Yet electrolysis at constant current is the oldest voltammetric method, as its origin can be traced to the work of Weber (*27*) in 1879. The method was applied about 1900 by Sand (*22*), Cottrell (*4*), and Karaoglanoff (*16*) to the verification of Fick's law of diffusion and to the determination of diffusion coefficients. A similar application was recently made by von Stackelberg and coworkers (*25*). The method was applied by Butler and Armstrong (*2, 3*) in electrochemical kinetics, but it is only recently that the potentialities of the constant current method in analytical chemistry were first realized, thanks to the work of Gierst and Juliard (*12, 13*). Contributions to the theory of voltammetry at constant current, which recently had not been fully developed, were made by Delahay and coworkers (*1, 6–9*).

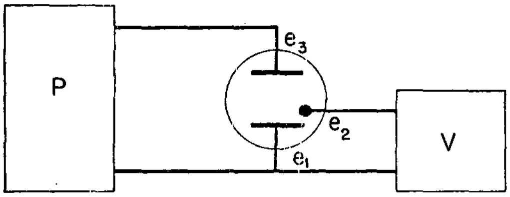

Figure 1. Schematic diagram of apparatus for voltammetry at constant current

Electrolysis at constant current can be performed with the instrument schematically represented in Figure 1. The cell is connected to a power supply, *P*, which controls the current. The potential of the working electrode e_1 is measured against a reference electrode, e_2, by means of instrument *V*, which is generally a pen-and-ink recorder or a cathode-ray oscillograph. In general, the resulting potential-time curves can be analyzed quantitatively when the following conditions are fulfilled: The electrolysis current is constant, and the solution is not stirred and contains a large excess of supporting electrolyte—i.e., diffusion is the sole mode of mass transfer of the electrolyzed species. Only linear diffusion is considered here.

A qualitative analysis of potential-time curves is described for the simple case of the reduction of cadmium ion on a mercury pool electrode. This process is reversible, and the potential of the mercury pool electrode can be expressed by the Nernst equation. If one assumes that the activity of mercury is equal to unity (dilute amalgam), one has

$$E = E^0 + \frac{RT}{2F} \ln \frac{f_{Cd^{++}}\, C_{Cd^{++}}}{f_{Cd}\, C_{Cd}} \qquad (1)$$

where E^0 = the standard potential for the cadmium-amalgam electrode
f = activity coefficient
C = concentration at the electrode surface

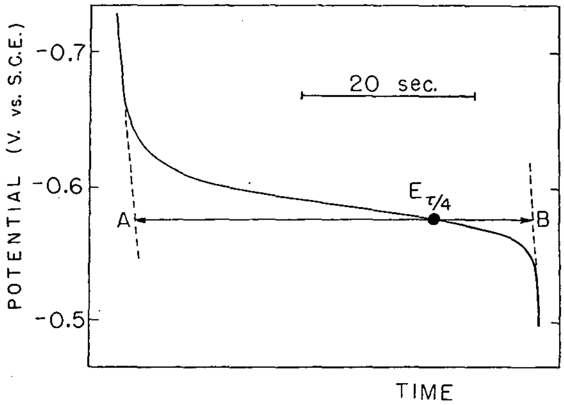

Figure 2. Potential-time curve for reduction of 4*mM* cadmium(II) in 1*M* potassium nitrate

Mercury pool electrode. Increasing time from right to left
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It is seen from Equation 1 that variations of potential during electrolysis are determined by the ratio $C_{Cd^{++}}/C_{Cd}$. The concentration of cadmium ion at the electrode surface decreases during electrolysis because of the consumption of this ion in the electrode process. Conversely, the concentration of cadmium in mercury increases since cadmium is continuously produced. As a result, the potential becomes more cathodic as the electrolysis proceeds (Figure 2). Initially there is no cadmium in mercury, and the potential of the mercury pool electrode according to Equation 1 should be $+\infty$. Actually, this equation does not hold when cadmium is not present, but at any rate the potential is markedly anodic. As the electrolysis proceeds, the concentration of cadmium ions at the electrode surface decreases and is finally equal to zero; the potential according to Equation 1 should then be $-\infty$. Actually, the potential becomes so cathodic that some process other than the reduction of cadmium ion occurs—for example, the supporting electrolyte is reduced.

The time τ at which the concentration of electrolyzed species is equal to zero at the electrode surface is called transition time according to a suggestion of Butler and Armstrong (*2, 3*). This time is represented by segment *AB* in Figure 2. The square root of the transition time is generally proportional to the concentration of analyzed substance, and consequently the constant current method has some potentialities in analytical chemistry. Determinations can be made over a wide range of concentrations —10^{-6} to $10^{-2}M$—with good precision (possibly less than 1 to 2% error).

The foregoing comments concern electrolysis at constant current, but methods in which the current varies in a known fashion during electrolysis can be conceived. Indeed, such a method in which the current is a sinusoidal function of time was developed by Heyrovský (*15*). Valuable information on electrochemical

Table I. Fundamental Relationships

Process	Equation		Investigators
Process without kinetic complication	$\tau^{1/2} = \frac{\pi^{1/2}\, n\, FD^{1/2}\, C^0}{2i_0}$	(2)	Sand (*22*)
Reversible process, reaction products soluble	$E = E_{\tau/4} + \frac{RT}{nF} \ln \frac{\tau^{1/2} - t^{1/2}}{t^{1/2}}$	(3)	Karaoglanoff (*16*)
	$E_{\tau/4} = E^0 + \frac{RT}{nF} \ln \frac{f_O\, D_R^{1/2}}{f_R\, D_O^{1/2}}$	(4)	
Irreversible process involving single rate-determining step	$E = E_i + \frac{RT}{\alpha n_a F} \ln \left[1 - \left(\frac{t}{\tau}\right)^{1/2}\right]$	(5)	Delahay and Berzins (*7*)
	$E_i = \frac{RT}{\alpha n_a F} \ln \frac{nFk^0C^0}{i_0}$	(6)	
Consecutive processes	$(\tau_1 + \tau_2)^{1/2} - \tau_1^{1/2} = \frac{\pi^{1/2}\, n_2 F D_2^{1/2} C_2^0}{2\, i_0}$	(7)	Berzins and Delahay (*1*)
Stepwise processes	$\tau_2 = \tau_1 \left[\left(\frac{n_2}{n_1}\right)^2 + 2\,\frac{n_2}{n_1}\right]$	(8)	Berzins and Delahay (*1*)

kinetics can be obtained by this method, but the quantitative treatment of potential-time curves would be far more difficult than for the constant current method. Analytical applications of Heyrovský's method are also possible, although the method is more useful in qualitative analysis than in quantitative determinations (*14*).

The theory of voltammetry at constant current is discussed here, while applications are described by Reilley, Everett, and Johns (*20*). Fundamental relations are listed in Table I. The notations are as follows:

O, R = substances involved in electrode process
E = electrode potential, volts
t = time, seconds
τ = transition time, seconds
C^0 = bulk concentration, moles per ml.
i_0 = current density in amperes per sq. cm.
n = number of electrons involved in over-all electrode process
α = transfer coefficient for electrode process
k^0 = rate constant for an irreversible process, in cm. per second and at $E = 0$ (*vs.* normal hydrogen electrode)
R = gas constant
T = absolute temperature
F = the faraday
$2.3\ RT/F$ = 0.059 volt at 25°
π = 3.14

TRANSITION TIME

The concentration of electrolyzed substance at the electrode surface is equal to zero at the transition time. Hence, this concentration must be determined to obtain the transition time. The derivation was carried out by Weber (*27*), but his value of τ is in the form of an infinite series which is rather cumbersome to use. A more elegant solution was proposed by Sand (*22*), who derived Equation 2 in Table I. Sand solved Fick's equation for linear diffusion for the proper initial and boundary conditions. The boundary condition is obtained by expressing the current as constant—i.e., the flux of electrolyzed substance at the electrode surface is constant. If C_O represents the concentration of electrolyzed substance, the boundary condition is ($t > 0$)

$$D_O(\partial C_O/\partial x)_{x=0} = i_0/nF \tag{9}$$

where x is the distance from the electrode. The initial condition is $C_O = C^0$ if there is no gradient of concentration before electrolysis. The concentration for $x = 0$ is

$$(C_O)_{x=0} = C^0 - \frac{2\, i_0\, t^{1/2}}{\pi^{1/2}\, nFD_O^{1/2}\, C^0} \tag{10}$$

From Equation 10 one deduces that C decreases continuously during electrolysis and that $C = 0$ at the transition time $t = \tau$ (compare with Equation 2). The decrease in C is more pronounced as the current density is increased; the electrolyzed substance is consumed more rapidly at the electrode, and the transition time is shorter.

It is seen from Equation 2 that the square root of the transition time is proportional to the concentration of electrolyzed species and inversely proportional to current density. Hence, the product $i_0\tau^{1/2}$ is independent of current density for given conditions of electrolysis, and transition times obtained at different current densities can be correlated by Equation 2. The validity of this conclusion has been verified by several authors (*12, 13, 16, 21, 22, 25*). Equation 2 is not applicable when there are kinetic complications (see below).

Since $\tau^{1/2}$ is inversely proportional to i_0, the transition time can be adjusted over a wide range by proper selection of the current density. In practice, values of τ smaller than 1 millisecond cannot be measured precisely because of the effect of the residual current which is discussed below. The upper limit of τ depends on the care which is taken to avoid vibrations of the cell and concomitant interference of convection with diffusion. The authors have measured repeatedly transition times of the order of 1 minute with a maximum deviation of approximately 1%. These measurements, which are discussed by Reilley and coworkers (*20*), were made on the second floor of a building (with air-conditioning machinery in the building) without any special precautions. Possibly much longer transition times could be measured with precision although there is no real need for such measurements.

It is seen from Equation 2 that the precision of the transition time for a given concentration depends on factors D and i_0. The value of the diffusion coefficients, D, can be kept constant in comparative measurements by using the same concentration of supporting electrolyte and by maintaining the temperature constant within, say, ±0.1° C. The precision of the current density depends on the errors made in the measurement of the current intensity and on the reproducibility of the electrode area. Since current intensities can easily be controlled within ±0.1%, it is the reproducibility of the electrode area which is determinative. At concentrations of the order of 10^{-2} to $10^{-3}M$ the maximum deviation of τ does not exceed approximately 1 to 2%, and the maximum error of temperature is 0.5 to 1% ($\tau^{1/2}$ is proportional to temperature, ° C.). However, the current density at the electrode surface will generally not be the same over the whole electrode area, unless special care is taken in the design of the cell. As a result, the calculated current density (current per area of electrode) differs somewhat from the actual current density in the area of the working electrode whose potential is measured against a reference electrode. Because of this uncertainty about current densities, concentration determinations based on the application of Equation 2 are not advisable and calibration of the cell is preferable. Thus, the current density in Equation 2 should be multiplied by a factor β to take into account the variation in current density; β is generally smaller than unity because of the shielding of the working electrode by the tip of the reference electrode. The value of the transition time can be calculated with relatively good precision by introducing in this modified Equation 2 diffusion current constants available in the polarographic literature (*19*).

POTENTIAL-TIME CURVES FOR REVERSIBLE PROCESSES

The potential of the working electrode for a reversible process can be calculated from the Nernst equation. The concentration of the oxidant at the electrode surface is given by Equation 10, and the concentration of the reductant, which is derived by solving Fick's equation, is

$$(C_R)_{x=0} = \left(\frac{D_O}{D_R}\right)^{1/2} [C^0 - (C_O)_{x=0}] \tag{11}$$

By introducing $(C_R)_{x=0}$ and $(C_O)_{x=0}$ in the Nernst equation, one obtains Equation 3 of Table I which was derived by Karaoglanoff (*16*). This equation holds for cases in which R is soluble either in solution or in the electrode (amalgam formation).

It is seen from Equation 3 that a plot of log $\{(\tau^{1/2} - t^{1/2})/t^{1/2}\}$ *vs.* E should yield a straight line whose reciprocal slope is 2.3 RT/nF—e.g., $0.059/n$ at 25° C. Karaoglanoff (*16*) attempted to verify this conclusion experimentally. Transition times were too long (several hours), and discrepancies were observed between theory and experiment on account of interference by convection. Turner and Winkler (*26*) were not successful either, because they failed to work in the presence of a large excess of supporting electrolyte; the effect of migration cannot be neglected under these conditions. More reliable results were obtained by Delahay and Mattax (*8*) who used a fast pen-and-ink recorder (Brown, 1 second full scale deflection, chart speed of 480 inches per hour) and selected values of τ of the order of 1 minute. The experimental slopes of the logarithm diagrams for the reduction of thallium(I), cadmium(II) on a mercury pool electrode and the reduction of ferricyanide on a platinum electrode were essentially those predicted by theory. The same conclusion was reached by Reilley and coworkers (*20*). An example of logarithmic plot is shown in Figure 3 for processes for which $n = 1$, 2, and 3.

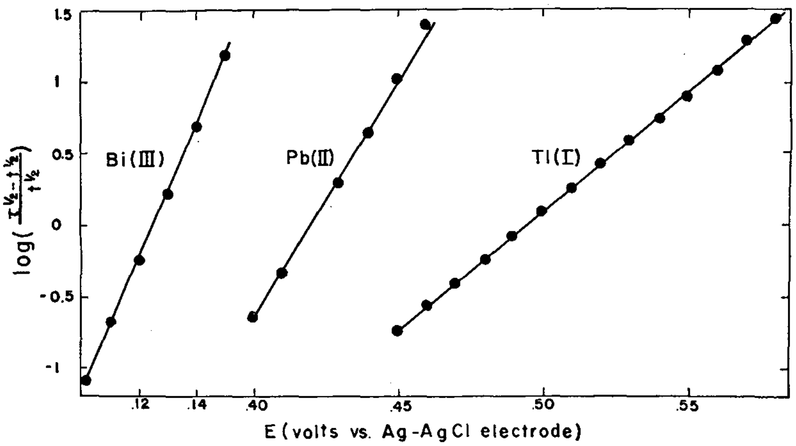

Figure 3. Plots of log$\{(\tau^{1/2} - t^{1/2})/t^{1/2}\}$ *vs.* E for reversible processes

Mercury pool electrode. 0.1mM solution of reducible species [data supplied by Reilley *et al.* (*20*)]. 0.01M potassium nitrate for thallium and lead, 1N sulfuric acid for bismuth

One deduces from Equation 3 that the potential E has the value $E_{\tau/4}$ (Equation 4) at one fourth of the transition time. Potential $E_{\tau/4}$ might therefore be called the quarter-time potential or, as Reilley *et al.* suggested (*20*), the quarter-wave potential. The potential $E_{\tau/4}$ is approximately equal to the standard potential E^0 since the ratio $f_O\ D_R^{1/2}/f_R D_O^{1/2}$ in Equation 4 is close to unity. If a mercury electrode is utilized, $E_{\tau/4}$ is equal to the polarographic half-wave potential as was pointed out by Delahay and Berzins (*7*); Equation 4 is identical to the formula for the polarographic half-wave potential (*17*). The validity of this conclusion was demonstrated by Delahay and Mattax (*8*) who reported values of $E_{\tau/4}$ which do not differ from the $E_{1/2}$ by more than 0.01 volt for the reduction of cadmium(II), thallium(I), and ferricyanide. The $E_{\tau/4}$ potentials in Figure 3 are also in good agreement with the corresponding half-wave potentials (*17*).

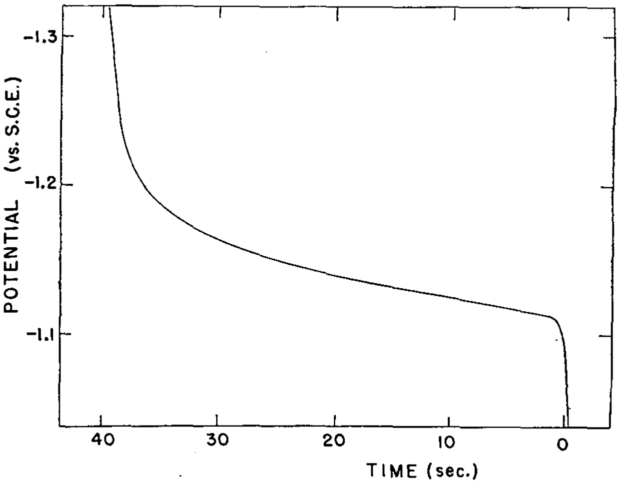

Figure 4. Potential-time curve for the reduction of 4mM potassium iodate in 1M sodium hydroxide

Equation 3 is identical to the equation for the corresponding polarographic wave, except that i_d and i are replaced by $\tau^{1/2}$ and $t^{1/2}$, respectively. Likewise, Equation 2 for the transition time is the counterpart of the Ilkovič equation in polarography. These equations for the constant current method, which unfortunately remained buried in the literature for so long, were derived approximately 30 years before the corresponding formulas in polarography were established by Heyrovský and Ilkovič.

POTENTIAL-TIME CURVES FOR IRREVERSIBLE PROCESSES

No assumption about the degree of irreversibility of the electrode process is introduced in the derivation of Equation 10, and consequently Equation 2 for the transition time is also valid for irreversible process. The potential of the working electrode was derived by Delahay and Berzins (*7*) by equating the current to the rate of electron transfer. Thus

$$i_0 = nF(C_O)_{x=0}\, k^0 \exp.\left(-\frac{\alpha n_a F E}{RT}\right) \qquad (12)$$

Equation 12 is written on the assumptions that the influence of the backward process can be neglected and that the kinetics of electron transfer is controlled by a single rate-determining step; these hypotheses are valid for numerous irreversible processes. By rearranging terms in Equation 12 and introducing the transition time from Equation 2, one obtains Equation 5 for irreversible electrode processes.

It follows from Equation 5 in Table I that the potential, immediately after time $t = 0$, varies abruptly at the potential E_i defined by Equation 6. This is essentially the case for experimental potential-time curves, as can be seen from Figure 4. One also deduces from Equation 5 that a plot of log $\{1 - (t/\tau)^{1/2}\}$ *vs.* E should yield a straight line whose reciprocal slope is 2.3 $RT/\alpha n_a F$ (Figure 5). This conclusion was verified experimentally by Delahay and Mattax (*8*).

The shape of irreversible potential-time curves is determined by the value of the product αn_a—i.e., by the transfer coefficient α and by the number of electrons n_a involved in the activation step. The transfer coefficient α is smaller than unity and n_a is in general equal to 1 or 2. As a result, potential-time curves for irreversible processes are more drawn out in the scale of potentials than in the case of reversible processes. For example, the curve of Figure 4 for iodate should have been very steep, had the process been reversible ($n = 6$).

The potential E_i defined by Equation 6 depends on the current density, and consequently current-potential curves for irreversible processes can be shifted somewhat in the scale of potentials by adjustment of the current density. This might be interesting in the case of overlapping curves (one reversible and one irreversible process), but the usefulness of this procedure is limited by the small amplitude of the shift of potential (logarithmic dependence, see Equation 6). The value of E_i observed with a mercury electrode can be predicted from polarographic half-wave potentials as follows:

The polarographic half-wave potential can be deduced from the treatments of irreversible polarographic waves which have been developed by various authors (*5, 10, 11, 24*) for linear diffusion and by Koutecky (*18*) for diffusion at an expanding sphere. One deduces from the latter author's treatment that the following condition is fulfilled at the half-wave potential

$$\frac{k^0 t_p^{1/2} \exp.\left(-\dfrac{\alpha n_a F\ E_{1/2}}{RT}\right)}{D^{1/2}} = 0.76 \qquad (13)$$

where t_p is the drop time. Equation 13 is written for cathodic processes on the assumption that the backward process can be neglected and that the maximum current during drop life is measured. Equation 13 virtually holds for average currents. By deducing k^0 from Equation 13 and introducing the resulting value in Equation 6 for E_i, one obtains, after simple transformations,

$$E_i = E_{1/2} + \frac{RT}{\alpha\ n_aF} \ln \frac{2 \times 0.76}{\pi^{1/2}} \left(\frac{\tau}{t_p}\right)^{1/2} \qquad (14)$$

or for 25°

$$E_i = E_{1/2} + \frac{0.059}{\alpha n_a} \log 0.86 \left(\frac{\tau}{t_p}\right)^{1/2} \qquad (15)$$

Since the order of magnitude of αn_a is one, it follows from Equation 15 that the potential E_i is not very different from $E_{1/2}$.

Potentials $E_{1/2}$ calculated from Equation 15 on the basis of data obtained by the constant current method are in good agreement with experimental half-wave potentials as was demonstrated by Delahay and Mattax (*8*).

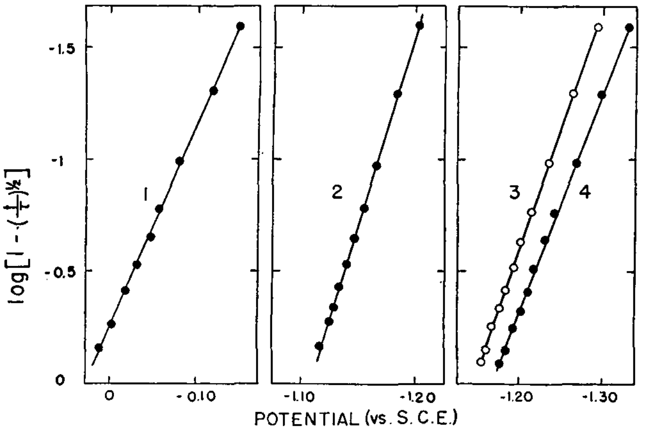

Figure 5. Plots of log $\{1 - (t/\tau)\}^{1/2}$ *vs.* E for irreversible processes

Mercury pool electrode
1. Oxygen in acetate buffer of pH 4.6
2. 4*mM* iodate in 1*M* sodium hydroxide
3. 4*mM* nickel(II) in 1*M* potassium chloride
4. 4*mM* cobalt(II) in 1*M* potassium chloride

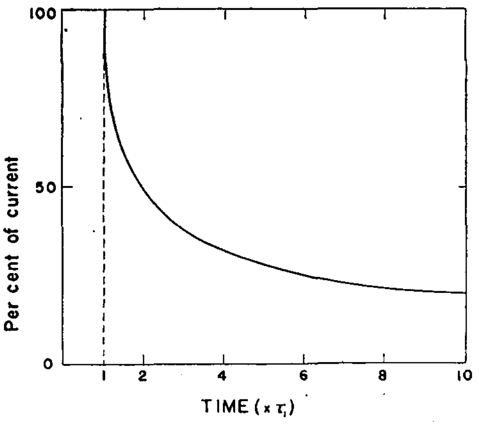

Figure 6. Contribution of O_1 to total current

Case of two consecutive electrode processes

CONSECUTIVE ELECTRODE PROCESSES

Two substances O_1 and O_2 are reduced (oxidized) at somewhat different potentials, and the resulting curve exhibits two steps. The transition time τ_1 for the first step can be calculated by Equation 2, but this is not the case for the second step. The potential of the working electrode after the transition time τ_1 adjusts itself at a value at which substance O_2 is reduced. However, substance O_1 continues to diffuse toward the electrode at which it is reduced. Because of this contribution of substance O_1 to the current, the concentration of O_2 at the electrode surface decreases less rapidly than it would have, had substance O_1 not been present. The contribution of substance O_1 to the total current is plotted against time in Figure 6 which shows that the contribution of O_1 is still important even when t is several times τ_1. This diagram was given by Berzins and Delahay (*1*) in their derivation of the equation for the transition time τ_2. This derivation is involved but the result is expressed by the simple Equation 7 in Table I. Equations 2 and 7 are identical, except that it is now the quantity $(\tau_1 + \tau_2)^{1/2} - \tau_1^{1/2}$ which is proportional to concentration instead of the square root of the transition time. The validity of Equation 7 was verified for mixtures of cadmium and zinc ions (*1*).

The increase in transition time τ_2 resulting from the contribution of substance O_1 can be judged by considering the case in which the diffusion coefficients and bulk concentrations of substances O_1 and O_2 are the same. The transition times, τ_1 and τ_2, measured in separate electrolyses are the same, while $\tau_2 = 3\tau_1$ when both substances are present in solution. This increase in the value of τ_2 is advantageous when concentration C_2^o is much smaller than C_1^o. Furthermore, there is also the possibility of decreasing manually or automatically the current density after the first transition time is reached. The transition time τ_2 can then be adjusted at a value comparable to τ_1 although C_2^o is much smaller than C_1^o, but Equation 7 must be modified to take into account the change in current density. Thus

$$\left\{\tau_1 \left[\frac{(i_0)_1}{(i_0)_2}\right]^2 + \tau_2\right\}^{1/2} - \tau_1^{1/2} \left[\frac{(i_0)_1}{(i_0)_2}\right] = \frac{\pi^{1/2} n_2 F\ D_2^{1/2} C_2^o}{2(i_0)_2} \qquad (16)$$

where $(i_0)_1$ and $(i_0)_2$ are the current densities for the first and second step, respectively.

The case in which more than two substances are involved has not been treated. It has been suggested by Reilley *et al.* (*20*) that Equation 7 might be applicable in the form

$$(\tau_1 + \tau_2 \ldots + \tau_p)^{1/2} - (\tau_1 + \tau_2 \ldots + \tau_{p-1})^{1/2} = \frac{\pi^{1/2}\, n_p F\ D_p\ C_p^o}{2\, i_0} \qquad (17)$$

Obviously Equation 17 yields Equations 2 and 7 when p is made equal to 1 and 2, respectively. Equation 17 seems also valid for larger values of p as indicated by experimental results (*20*), but the derivation of this equation appears very arduous even for $p = 3$. Cases in which p is larger than 2 or 3 have little practical value in view of the accumulation of errors in the determination of consecutive transition times.

STEPWISE PROCESSES

Consider the stepwise reduction (oxidation) of a substance O in two steps involving n_1 and n_2 electrons, respectively. Only the transition time for the first step can be calculated by Equation 2. After time τ_1 substance O continues to diffuse toward the electrode where it is reduced (oxidized) in a process involving $n_1 + n_2$ electrons. Furthermore, the product formed during the first step of electrolysis diffuses back toward the electrode where it is reduced (oxidized) with the consumption of n_2 electrons. The value of τ_2 was derived by Berzins and Delahay (*1*) who reported Equation 8. This formula yields $\tau_2 = 3\ \tau_1$ for

$n_1 = n_2$ and $\tau_2 = 8\tau_1$ for $2\,n_1 = n_2$. Equation 8 was verified experimentally (*1*) for the reduction of oxygen ($\tau_2 = 3\,\tau_1$) and uranyl ion ($\tau_2 = 8\,\tau_1$).

COMPLICATIONS RESULTING FROM KINETIC AND ADSORPTION PROCESSES

The transition time for numerous processes is accounted for by Equation 2. However, there are exceptions for which the product $i_0\tau^{1/2}$ decreases with increasing current densities, while this product should be constant according to Equation 4. This effect is shown in Figure 7 where τ_k is the observed transition time and τ_d is the transition time which would have been observed had there not been any departure from Equation 2. This abnormal behavior, which was first observed by Gierst and Juliard (*12, 13*) for the reduction of cadmium cyanide, was explained by these authors by assuming that the complex undergoes some chemical reaction before the electron transfer process. Such "kinetic" complications have been reported in polarography for certain substances, although not for complex ions in general; the polarographic behavior of the complexes of Figure 7 is normal—i.e., limiting currents obey the Ilkovič equation. Thus kinetic effects which can be detected by voltammetry at constant current do not necessarily appear in polarography. Gierst and Juliard (*12, 13*) calculated the transition time by introducing the reaction layer thickness, but their results are approximate because the concept of reaction layer thickness is somewhat artificial. A rigorous treatment was given by Delahay and Berzins (*1*) who reported

$$\left(\frac{\tau_k}{\tau_d}\right)^{1/2} = 1 - \frac{\pi^{1/2}}{2\tau_d^{1/2}\,K\,k_b^{1/2}} \qquad (18)$$

where k_b = rate constant for the backward chemical reaction preceding the electron transfer and K = equilibrium constant for the chemical process. Equation 18 holds for values of $k_b^{1/2}\,\tau_k^{1/2}$ larger than 2 and for first-order chemical reactions [for other conditions see (*7*)]. It follows from Equation 18 that Equation 2 can be applied, provided that the quantity $\tau_d^{1/2}\,K\,k_b^{1/2}$ is much larger than unity. This will be the case when the transition time τ_d is sufficiently long—i.e., when the current density is low enough (see Equation 2). Since $\tau_d^{1/2}$ is inversely proportional to current density, one can, in principle, find a value of i_0 so large that $(\tau_k/\tau_d)^{1/2} < 1$. This current density may in some cases be in the range usually selected, and a serious error may arise from application of Equation 2 in the conversion from one current density to another. Therefore, it is essential to verify that the product $i_0\,\tau^{1/2}$ is actually independent of current density before converting, by means of Equation 2, transition times obtained at different current densities.

Another cause of error may result from the adsorption of substances such as gelatin on the electrode. Gierst and Juliard (*12, 13*) studied this effect in the case of the dropping mercury electrode. Potential-time curves were recorded in a small fraction of drop life, and consequently the mercury drop behaved practically as a stationary electrode. The addition of gelatin (0.001% or even less) caused an appreciable decrease in the transition time. Observations on the behavior of the mercury pool electrode would be of great interest.

INFLUENCE OF DOUBLE LAYER

Since the potential of the working electrode varies during electrolysis, the surface charge density of electricity at the interface electrode-solution varies, and a fraction of the current through the cell is used in the charging or discharging of the double layer. As a result, potential-time curves are distorted, as can be seen from Figure 2; the segments traced in dashed lines in this diagram should be essentially vertical, had there been no double layer effect. The quantity of electricity used in the charging (or discharging) of the double layer between two potentials E_1 and E_2 is $Q = i_0\tau_c$ where τ_c is the time elapsed from E_1 to E_2 in the absence of any electrochemical reaction. Furthermore, one has $i_0\,\tau^{1/2} = P$ where τ is the transition time and P represents various terms in Equation 2. It follows from these equations that

$$\frac{\tau_c}{\tau} = \frac{Q\,i_0}{P^2} \qquad (19)$$

which shows that the ratio τ_c/τ increases with i_0. Hence, the distortion of potential-time curves is more pronounced when the current density is increased. Since P is proportional to the bulk concentration C^0 of electrolyzed substance, the distortion of potential-time curves is more accentuated as the bulk concentration of electrolyzed substance is decreased. In practice it is observed that even with solutions as concentrated as $5 \times 10^{-2}M$ the distortion of potential-time curves becomes very pronounced when the current density is so large that the transition times are shorter than 1 millisecond.

Because of the distortion of potential-time curves, standardized methods must be adopted in the graphic determination of transition times. The method illustrated in Figure 8, *A*, which is inspired from polarographic practice, was recommended by Delahay and Berzins (*7*). It is applicable when the segments *AH* and *CF* are almost parallel. One constructs $AB = {}^1/_4\,AC$ and $HG = {}^1/_4\,HF$; line *BG* intersects the potential-time curve at $E_{\tau/4}$, and *KL* represents the transition time. When lines *AK* and *CF* are markedly diverging, the construction of Figure 8, *B*, is to be preferred. The transition time is represented by segment *KL*; *L* is the point at which the potential-time curve becomes essentially linear. The construction of Figure 8, *A*, is justified for reversible processes in view of the definition of potential $E_{\tau/4}$. This is not the case for irreversible processes, but any other graphic construction would be just as empirical when the segments *AH* and *CF* are not parallel.

Electrolysis times can obviously be plotted along the ordinate axis instead of the abscissa axis; potential-time curves have then the same appearance as polarographic waves.

MISCELLANEOUS PROCESSES

In addition to the foregoing theoretical developments, the following problems have been solved: transition time in electrolysis with reversal of current after the transition time (*1*); equation of potential-time curve for the second step in the electrolysis of two substances (*1*); kinetic processes in which the product of electrolysis undergoes a chemical reaction (*9*); catalytic processes involving a first order chemical reaction (*9*); anodic oxidation of metals with formation of a sparingly soluble substance or a complex ion (*9*); transition time in the case of mixed control by linear diffusion and electric migration (*9*); and transition time for spherical diffusion (*9*).

POTENTIALITIES OF CONSTANT CURRENT METHOD

Voltammetry at constant current has interesting features which, in some cases, may render the method more advantageous than

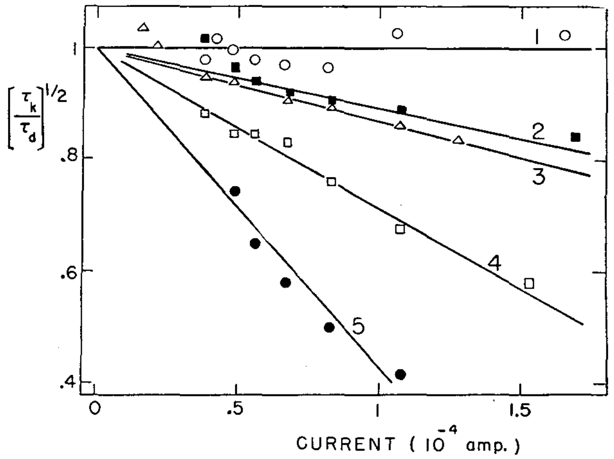

Figure 7. Variations of $(\tau_k/\tau_d)^{1/2}$ with current for the reduction of various complex ions

1. 1*mM* zinc, 2*M* ammonium hydroxide, 2*M* ammonium chloride
2. 1*mM* cadmium, 1*M* potassium iodide, 1*M* potassium nitrate
3. 1*mM* nickel(II), 1*M* potassium cyanide, 1*M* potassium nitrate
4. 2*mM* cadmium, 0.5*M* sodium tartrate, 1*M* sodium nitrate
5. 1*mM* cadmium, 0.1*M* dipyridyl, 1*M* potassium nitrate

Electrode area, 0.02 sq. cm., approximately (dropping mercury electrode)
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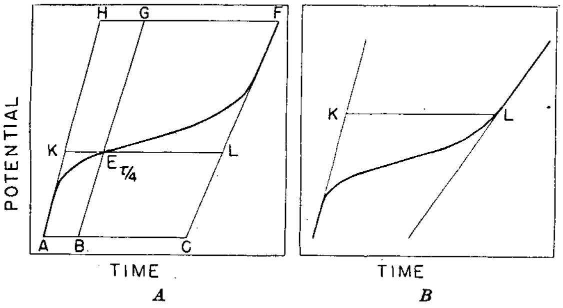

Figure 8. Graphic determination of transition time

other electrochemical methods of analysis and in particular conventional polarography: good precision; no fluctuation in the measured quantity (in contrast with the fluctuations of the diffusion current in polarography); possibility of determining concentrations as low as $10^{-6}M$; possibility of using solid or mercury electrodes; possibility of analyzing certain two-component systems without preliminary separation (see above); and utilization of instruments in which the electrolysis can be stopped automatically at the transition time, as was demonstrated by the work of Sartiaux (*23*). Two disadvantages should be mentioned: no steady reading is observed; and consumption of mercury is larger than in polarography. Finally, voltammetry at constant current is a useful tool in electrochemical kinetics (*6–8*).

Voltammetry at constant current appears to be a promising new analytical tool. In future work it might be worth while to designate this method by the term "chronopotentiometry," which is less cumbersome than "voltammetry at constant current."

ACKNOWLEDGMENT

This investigation was sponsored by the Office of Naval Research. The authors take pleasure in acknowledging their indebtedness to this organization.

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RECEIVED for review May 24, 1954. Accepted August 4, 1954. Presented before the Division of Analytical Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y. Apparatus for application of the method will be made available by Sargent & Co., Chicago, Ill.

Voltammetry at Constant Current

Experimental Evaluation

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The feasibility of voltammetry at constant current (chronopotentiometry) as an analytical method is elucidated in the cathodic reduction and anodic oxidation of simple ions at platinum and mercury pool electrodes where transition times vary between 4 and 60 seconds. Interpretation of potential-time curves yields successful analytical results for solutions of single and mixed ions in concentrations of 5×10^{-5} to $1 \times 10^{-2}M$. Theoretical and experimental comparisons to other polarographic methods are discussed. Several supporting electrolytes are examined, and practical apparatus and technique are described.

CLASSICAL polarography has in 20 years attained a position of unique importance in analytical chemistry. While there has been great progress in instrumentation and technique, the method is hampered by two characteristics of the dropping mercury electrode itself: The charging current of the growing drop is exceedingly large, and the pulsating nature of the measured current variable makes its evaluation difficult. These factors constitute a practical limitation to analytical polarography in lower concentration ranges where the diffusion currents encountered are small.

Several recent contributions to the polarographic field have been directed toward enhancing the sensitivity of the method by lessening the charging current of the system caused by an increasing electrode surface area. Stationary and moving platinum electrodes are advantageous in this respect, but lack the surface reproducibility and favorable hydrogen overvoltage of mercury. Mercury macroelectrodes have proved advantageous in oscillographic polarography but, because of the short scanning interval involved, the charging current density is relatively great (*5*).

From the analytical standpoint (especially for voltammetry at constant current, chronopotentiometry, where voltage-scan or transition times are made relatively long for accurate measurements), the quiet mercury pool electrode would seem ideally suited. Its surface is constant and reproducible, and its hydrogen overvoltage is high. The strictly linear diffusion problem encountered with a pool may be treated more rigorously than that of a growing spherical drop. While the surface area of the electrode has no theoretical influence upon the detectability of the electroactive species, a large pool is practically advantageous in being flatter and more reproducible. The pool used here is slightly less than 2 cm. in diameter, and platinum electrodes used for comparative studies are of similar area.

The measurement of transitory phenomena affords a unique analytical advantage: The combined ability to use quiet solutions (because a state of complete rest can be better reproduced than a state with a definite stirring action) and electrodes of constant area (to minimize charging current).

The transitory methods might be classified along the following pattern:

A. Application of potential to electrode-solution interface and measurement of resulting transitory current
   1. Potential constant with time
   2. Potential varies linearly with time
   3. Potential varies sinusoidally with time
   4. Potential varies as square-wave with time
   5. Potential with small superimposed alternating current potential varies linearly with time
   6. . . .
B. Application of current to electrode-solution interface and measurement of resulting transitory potential
   1. Current constant with time ("chronopotentiometry")
   2. Current varies sinusoidally with time
   3. . . .
C. Indirect application and measurement of current and/or potential
   1. Bridge methods
   2. . . .

Methods A 2 and B 1 have the advantages of covering a potential spectrum in a single trial and yielding results that may be interpreted in a relatively simple and straightforward manner. In addition, these two methods have properties which allow the distorting effect of the charging current to be minimized.

In chronopotentiometry, the charging time component varies inversely with the current density, whereas the transition time for the diffusion-controlled electrochemical reaction varies inversely with the square of the current density. Thus halving the current density will double the charging time, but will increase the transition time four-fold. The use of smaller current densities, necessary for very low concentrations, actually produces an enhancement of the response ratio of the system, and yields a great overall sensitivity. The ultimate limitation for increasing the response ratio by decreasing the current density occurs when the transition time becomes greater than approximately 1 minute. After about 1 minute, convection currents begin to contribute and abnormally large and erratic transition times are encountered.

Linear voltage-scan polarography, transient method A 2, has a similar advantage. The charging current is proportional to the scanning rate. Hence, for less distortion, lower scanning rates must be used, but this in turn causes the peak-current response to decrease. This decrease in peak-current response may be compensated to some extent by using more sensitive current recorders. The real limitation in decreasing the scanning rate is caused whenever the time required to scan the voltage from the foot of the first wave to the peak of the last wave exceeds about 1 minute. If longer times are employed, convection currents will begin to contribute and results will be erratic.

Chronopotentiometry has a definite advantage over method A 2 for systems containing more than one reducible species. In method A 2 the peak current for the second wave depends upon the concentration of the species yielding the first wave, the concentration of the species yielding the second wave, and the difference between the two half-wave potentials. In chronopotentiometry, the height of the second wave depends upon the concentrations of both species, but is independent of the difference between their half-wave potentials. Furthermore, in method A 2 as the time required for the total scan should not exceed about 60 seconds, the wider the separation of the half-wave potentials between the first and last waves, the greater will be limitation on the minimum rate of scan that can be tolerated. Thus two species with half-wave potentials very widely separated cannot be determined in as low concentrations as for two species with half-wave potentials closer together. No consideration of this type is necessary for chronopotentiometry. Streuli and Cooke (*5*) have used quiet mercury pools in the A 2 technique, and have obtained better sensitivity than that available in conventional dropping mercury electrode polarography; a comparison between their results and those obtained by chronopotentiometry is therefore of interest.

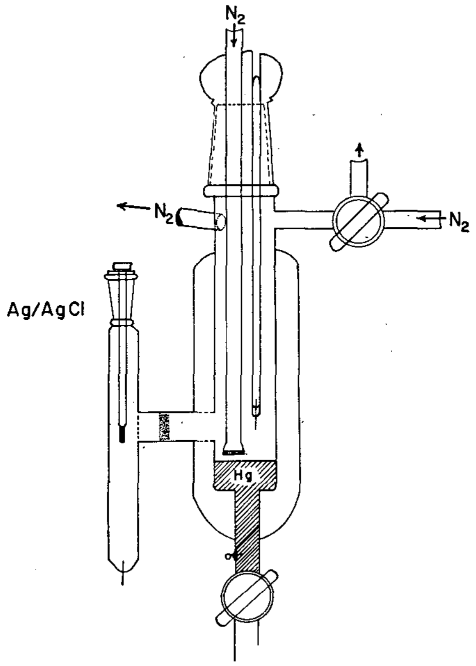

Figure 1. Chronopotentiometry cell

The present evaluation of chronopotentiometric analysis is based upon constant-current, diffusion-controlled electrolysis in unstirred solutions (*3*). Included are a study of single- and multiple-iron reductions at the mercury pool electrode; an investigation of several background electrolytes; and a comparison of theoretical and practical relationships among current, concentration, and transition time. Use of the platinum electrode is investigated and discussed for the sake of comparison with polarographic techniques.

APPARATUS AND MATERIALS

Electrolysis Cell. The cell used for chronopotentiometry is adapted from the design of Streuli and Cooke (*5*), and is illustrated in Figure 1.

It consists principally of a glass cylinder, 2 cm. in diameter, connected through a sintered disk to a silver-silver chloride reference electrode. A stopcock at the bottom of the cell permits easy draining, and an integral glass jacket provides for the circulation of constant-temperature water (25° ± 0.1° C.). The ground-joint top supports a small platinum wire electrode and a bubbling tube terminating in a sintered disk; the fritted glass increases the dispersion of nitrogen used in sweeping, thereby decreasing the time necessary for oxygen removal. A three-way stopcock may be adjusted for the initial sweeping of the solution, or alternatively for the passage of nitrogen above the solution during electrolysis.

The pool electrode consists of about 5 ml. of mercury, to which electrical contact is made by a platinum wire fused into the glass; the area of the pool was calculated to be 2.76 cm. A coating of Beckman Desicote on the inner surface of the cell prevents wetting of the glass by mercury or solution, thus maintaining a mercury-solution interface of constant area. An auxiliary cell top supports a platinum foil electrode for use in electrolysis at the platinum surface.

Delahay (*2*) developed the cell shown in Figure 2. He and Mamantov used it to obtain the very precise results in the electrolysis of iodate which are presented in Table I. The lower plug is of Lucite, and the mercury cavity is accurately machined on a lathe. Because Lucite is wet by neither mercury nor solution, the interface is constant and reproducible. The area of a mercury pool depends upon the potential because the extent of curvature around the edge of the pool depends upon the interfacial tension, which in turn depends upon the potential. The use of large pools minimizes this consideration. The reference electrode is very close to the mercury surface, thus minimizing the effect of ohmic potential differences caused by the electrolyzing current.

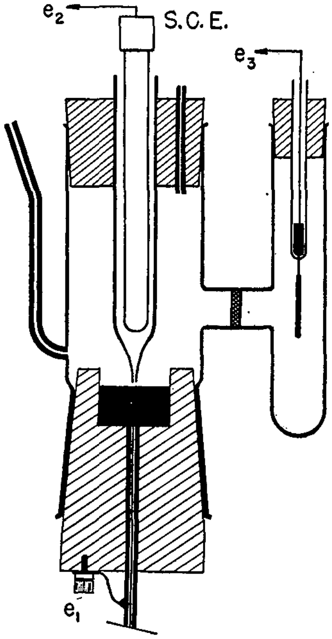

Figure 2. Chronopotentiometry cell (*2*)

Table I. Precision for Transition Time Determinations with Mercury Pool

10^{-3} Iodate, Moles/L. (1, 5)[a, b]		5×10^{-3} Iodate, Moles/L. (2, 5)[a, b]		10^{-2} Iodate, Moles/L. (3, 5)[a, b]		7×10^{-4} Cd^{++}, Moles/L. (4, 6)[a]	
τ sec.	Dev., %	τ, sec.	Dev., %	τ, sec.	Dev., %	τ, sec.	Dev., %
58.5	0	55.15	−0.09	56.9	−1.04	37.5	−1.8
58.4	−0.17	55.9	−1.27	57.65	0.26	37.7	−1.3
58.55	−0.08	55.45	0.45	57.5	0	38.9	1.8
57.9	−1.03	54.8	−0.73	57.35	−0.26	39.1	2.4
58.5	0	55.1	−0.18	57.8	0.52	38.5	0.78
58.6	0.17	55.3	0.18	57.5	0	38.3	0.26
58.95	0.77	55.35	0.27	57.85	0.61	37.3	−2.4
58.6	0.17	54.9	−0.54	57.0	−0.87	39.1	2.4
58.8	0.51	55.0	−0.36	57.5	0	38.0	−0.52
58.45	−0.08	55.05	−0.27	57.5	0	37.5	−1.8
58.5	0.30	55.2	0.43	57.5	0.36	38.2	1.55

[a] 1. Current, 1.057 ma.
2. Current, 5.324 ma.
3. Current, 10.33 ma.
4. Current, 0.136 ma.
5. Supporting electrolyte 1*M* NaOH, τ measured at −1.30 volts (S.C.E.)
6. Supporting electrolyte 0.1*M* KNO_3, τ measured at −0.578 volt (S.C.E.).

[b] Data supplied by Delahay and Mamantov (*3*).

Current Supply. Electrolysis current is supplied by a bank of five 45-volt dry batteries connected in series and tapped for various potentials as shown in Figure 3. A series of fixed resistances is inserted in the circuit in such a way that, by varying both voltage and resistance, a large variety of constant currents from 10^{-3} to 10^{-7} ampere may be obtained. Current values were calibrated, while electrolysis was in progress, by measuring the potential drop across a standard resistance by means of a potentiometer.

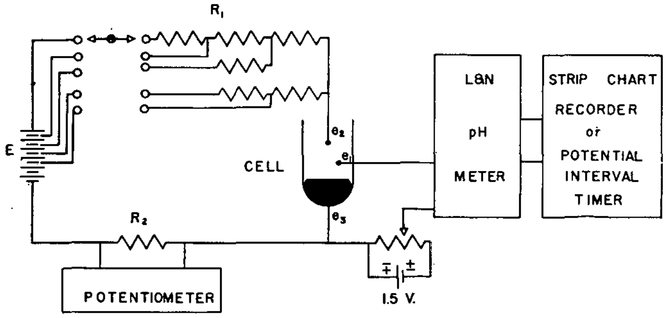

Figure 3. Circuit for chronopotentiometry

E. Series of 45-volt batteries with taps for 90–225 volts
R_1. Series of fixed swamping resistances
R_2. Standard resistance box
e_1. Reference half cell
e_2. Auxiliary electrode
e_3. Polarizing electrode
e_2–e_3. Polarizing current circuit
e_1–e_3. Potential indicating circuit
L&N pH meter, line-operated Model 7664

Measuring Circuit. The changing potential of the working electrode is followed during electrolysis by a direct-reading millivolt meter of high input resistance. A Leeds and Northrup Catalog 7664 pH meter serves the purpose, and its output was connected to a potentiometer-type strip-chart recorder. The Brown Electronik recorder with a full-scale scanning speed of 4.5 seconds and a chart speed of 8 inches per minute was used.

The changing potential may be followed with fair accuracy by watching the millivolt meter and timing the scanning interval between two points by means of a stop watch. Automatic interval timing could be attained by connecting the output of the meter of a trigger relay system, which would start and stop a timer at predetermined potentials.

Chemicals and Solutions. Chemicals used were of reagent grade and were analyzed as necessary by conventional procedures. Stock 0.1*M* solutions were prepared from the nitrates of thallium, copper, cadmium, bismuth, lead, and zinc; solutions of potassium ferrocyanide, ferric chloride, and ferrous chloride were also prepared, and all were diluted volumetrically to the concentrations used in electrolysis. It was found that water produced by a Barnsted Bantam ion exchange demineralizer contained less electro-reducible contamination than the available distilled water. The standard supporting electrolyte was potassium nitrate in either 0.1 or 0.01*M* concentration. Hydrochloric, nitric, and sulfuric acids were used where noted, as well as potassium chloride and buffered phosphate (pH 7.2).

Commercially obtained mercury was found to contain impurities which caused erratic results. Mercury was redistilled in the laboratory and freed of last traces of oil by allowing it to stand for a few hours in a grease-free buret.

Nitrogen used for sweeping was of the oxygen-free Seaford grade (Air Reduction Co.), and was used without further purification. It was saturated with water vapor at 25° and led to the cell through a system of Tygon tubing.

PROCEDURE

The cell is mounted on a special heavy stone ring stand, spring-supported and further cushioned with sponge rubber to minimize vibration. A pump provides circulation of constant-temperature water to the outer jacket of the cell.

Five milliliters of mercury are run into the cell to form the pool electrode. Alternatively, the pool is omitted and a platinum foil electrode, supported by an auxiliary cell top, is used. Approximately 20 ml. of solution are added, and the three-way stopcock is adjusted for bubbling of the liquid with nitrogen. After a bubbling period of 10 to 20 minutes, the gas flow is changed for sweeping of the upper portion of the cell, and the system is allowed to come to a complete state of rest before electrolysis is begun.

A double-pole single-throw switch could serve to start the electrolyzing current and the chart drive of the recorder simultaneously. However, more accurate results are obtained by allowing the chart drive to come up to full speed before the electrolyzing current is turned on. When no electroactive species is present, the cathode potential sweeps rapidly and continuously to the decomposition voltage of hydrogen ion, and the recorded potential-time curve, of the type shown in Figure 5, registers merely the charging of the double layer of the working electrode. If, on the other hand, an electroactive ion is present in the solution, the potential scans rapidly, as before, to the decomposition potential of that ion; then a potential holdup occurs as a diffusion layer is established. When a state of complete concentration polarization is attained, the scan continues, but at a reduced rate, producing a potential-time curve of the sort illustrated in Figure 4. The scanning rate for the upper plateau is decreased by virtue of the fact that the total current is now divided between the charging current and an important diffusion current resulting from the electrochemical reaction. Electrolysis is discontinued as soon as the upper portion of the curve is well defined; unless stated otherwise, the transition time is determined graphically as described by Delahay and Mamantov (*3*).

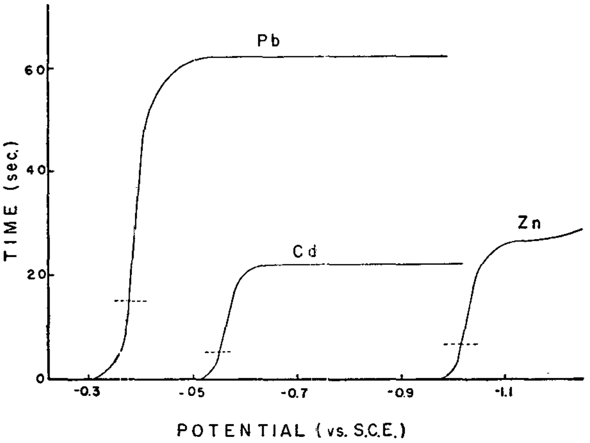

Figure 4. Potential-time curve for lead, cadmium, and zinc ions

Mercury pool using 0.1*M* KNO_3
Lead ion 1 × 10^{-3} *M*, 1.8 × 10^{-4} ampere
Cadmium ion 6.90 × 10^{-4} *M*, 1.8 × 10^{-4} ampere
Zinc ions 1.15 × 10^{-3} *M*, 2.73 × 10^{-4} ampere

The coupling circuit between the pH meter and recorder is so arranged that the full strip-chart width corresponds to a scan of 1 volt. A variable auxiliary 1.5-volt source, placed in series with the input to the meter, provides a bucking potential which allows any 1-volt scan within the range of −2.5 to +2.5 volts to be recorded.

Two runs per solution may be made with reproducible results. If more trials are attempted, the transition times decrease, owing to reduction of the bulk concentration in solution. The deviation becomes more pronounced for smaller currents and larger pools. The requirement of frequent changes of a relatively large quantity of mercury constitutes one of the most serious disadvantages of the method.

Transition times may be arbitrarily varied over wide limits by appropriate selection of electrolyzing currents. In practice, scans of less than 4 seconds are likely to be distorted by the lag in response of the recorder, while scans appreciably longer than 1 minute are more subject to the effects of convection in the solution (*4*). The diffusion layer extends further into solution with longer times and hence is more susceptible to distortion by vibration.

RESULTS

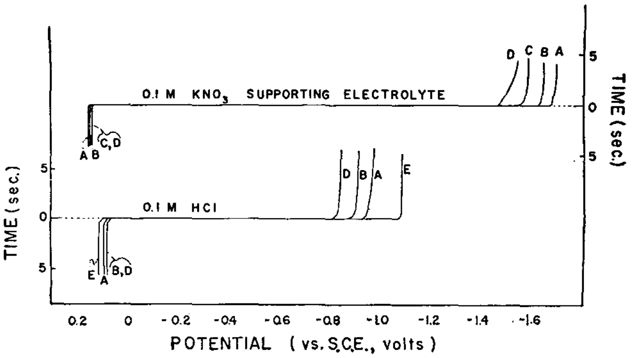

Figure 5. Effect of supporting electrolytes using mercury pool

A. 4.62 × 10^{-4} ampere
B. 2.30 × 10^{-4} ampere
C. 7.30 × 10^{-5} ampere
D. 3.28 × 10^{-5} ampere
E. 1.47 × 10^{-3} ampere

Background Electrolytes. Potential-time curves resulting from the electrolysis at several current densities of two supporting electrolytes are shown in Figure 5. With the mercury pool as the working electrode, solutions of 0.1 or 0.01*M* potassium nitrate are found to be the most useful. The generation of hydrogen does not begin until well beyond −1.0 volt (*vs.* S.C.E.) for currents of 3.5 × 10^{-5} ampere or larger, and hence would cause no interference in the reduction of many ions. The method of plotting is taken to resemble purposefully that of polarographic convention, in which cathodic currents are represented by a positive current scale and anodic currents by a negative one. As time is the measured variable in chronopotentiometry, cathodic transition times are plotted above the zero axis while anodic transition times are measured below it.

One effect must be realized which may be ascribed to the adsorption of oxygen or a thin layer of mercuric oxide on the mercury surface, since it persists despite prolonged sweeping of the solution with nitrogen. It was often noticed that an anomalous wave occurred during an electrolysis. A second run, after stirring with nitrogen or shaking to remove the diffusion layer, showed practically complete elimination of this wave. Also the transition times of ions reduced at more negative potentials than this anomalous wave were practically the same for the two trials. Had this anomalous wave been caused by an impurity in the solution, the height of the subsequent waves would have been enhanced, following the general theory of consecutive reductions discussed later. Bowden and Rideal (*1*) in their

Table II. Chronopotentiometric Analysis of Potassium Ferrocyanide Solution by Anodic Oxidation with Platinum Electrode

C, Moles/L. × 10^3	τ, Sec.	*I*, Amp. × 10^4	$I\tau^{1/2}$, Amp. Sec.$^{1/2}$ × 10^3	$\frac{I\tau^{1/2}}{C}$[a] × 10^{+3}
4	35.8	2.93	1.75	0.438
	15.4	4.40	1.73	0.433
	8.4	5.87	1.70	0.425
	5.3	7.47	1.72	0.430
6	35.8	4.40	2.64	0.440
	19.5	5.87	2.59	0.432
	12.2	7.47	2.61	0.435
	8.4	8.90	2.58	0.430
	4.5	11.9	2.52	0.420
8	37.9	5.87	3.60	0.450
	23.6	7.47	3.60	0.450
	16.1	8.90	3.57	0.446
	8.8	11.9	3.54	0.442
	5.6	15.0	3.56	0.445
10	35.5	7.47	4.44	0.444
	24.9	8.90	4.44	0.444
	13.5	11.9	4.37	0.437
	8.4	15.0	4.35	0.435
		Average		0.438
		Average deviation		0.0068
		% average deviation		1.55

[a] *C* in moles/ml.

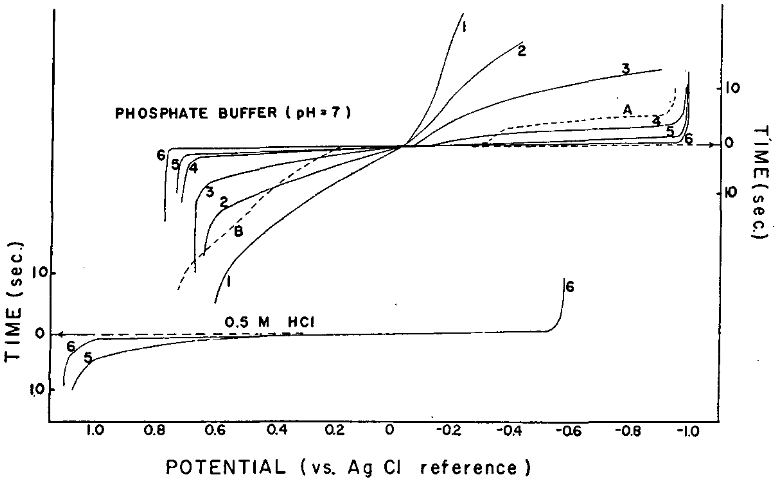

Figure 6. Effect of supporting electrolytes using platinum electrode

1. 1.50×10^{-6} ampere
2. 3.00×10^{-6} ampere
3. 6.12×10^{-6} ampere
4. 2.25×10^{-5} ampere
5. 4.50×10^{-5} ampere
6. 1.35×10^{-4} ampere

A. Wave due to adsorbed oxygen (1.35×10^{-4} ampere)
B. Wave due to adsorbed hydrogen (1.35×10^{-4} ampere)

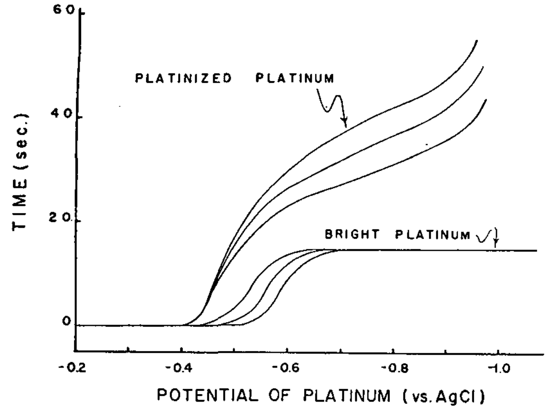

Figure 7. Potential-time curves for hydrogen evolution

$0.001M$ HCl in $0.1M$ KCl

study of the electrolytic behavior of thin films observed the same phenomenon.

Other supporting electrolytes examined with the mercury pool were potassium chloride, sodium acetate, and hydrochloric, nitric, and sulfuric acids. The working range of acid solutions is limited, of course, by the evolution of hydrogen.

Potassium chloride and buffered phosphate solutions offer a wide potential range for oxidation or reduction reactions at the platinum electrode with currents of 2×10^{-5} ampere per square centimeter or larger. Again, the effects of adsorbed films are evident at low currents; the dotted curves of Figure 6 show the effect of perhaps *A*, oxygen, and *B*, hydrogen films.

Figure 7 illustrates the potential-time curves for hydrogen evolution from a solution of $0.001M$ hydrochloric acid in $0.1M$ potassium chloride at bright and platinized platinum electrodes of almost the same projected geometrical area. Well-defined curves are obtained for the bright electrode; the quarter-wave potentials for the electrode varied considerably with its "cleanliness," a freshly-cleaned electrode evolving hydrogen at more positive potentials. An interesting result is that the same transition time was obtained for the various stages of cleanliness. This implies that cleaning decreased the hydrogen overvoltage of the electrode but did not alter its effective area.

With the platinized platinum electrode, the curves were neither as reproducible nor as well defined. The evolution of hydrogen from the hydronium ion as well as from water began at more positive potentials, as would be expected. Platinizing an electrode will increase its projected area only slightly, but its microscopic area will be increased manyfold. At low current densities, the diffusion layer will extend well beyond the surface roughness and the transition time will therefore depend more upon the projected area than upon the microscopic area. Thus the height of the hydrogen wave is increased relatively little by platinization. The steep slope of the curve in the region just beyond the transition period is attributed to the greatly increased microscopic area with its large double-layer capacitance and its increased ability to electrolyze the water.

Single-Ion Reductions. Delahay and Mamantov (*3*) have presented equations describing various chronopotentiometric processes. The key relationships for interpretation of potential-time curves from single-ion reactions are as follows: (symbols are those of Delahay and Mamantov; *I* is current and *A* is electrode area)

$$E = E_{1/4} + \frac{RT}{nF}\ln\frac{\tau^{1/2} - t^{1/2}}{t^{1/2}} \qquad (1)$$

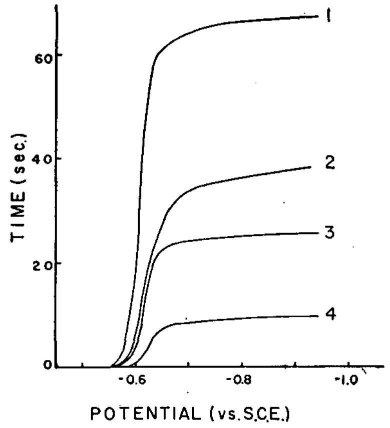

Figure 8. Effect of electrolyzing currents on potential-time curves

Mercury pool
Cadmium nitrate ($5 \times 10^{4}M$ in $0.1M$ KNO_3)

1. 6.00×10^{-5} ampere
2. 7.50×10^{-5} ampere
3. 9.00×10^{-5} ampere
4. 1.35×10^{-4} ampere

$$C^\circ = \frac{2i_0\tau^{1/2}}{nF\pi^{1/2}D^{1/2}} = \frac{2I\tau^{1/2}}{nF\pi^{1/2}D^{1/2}A} \qquad (2)$$

For a particular ion and a constant electrode area it may be seen from Equation 2 that:

$$\frac{I\tau^{1/2}}{C^\circ} = \text{a constant} \qquad (3)$$

If chronopotentiometry is to be of value as an analytical method, experimental data should be consistent with theoretical conclusions.

Equation 1 was verified in this laboratory by preparing from a wave a plot of $\log \frac{\tau^{1/2} - t^{1/2}}{t^{1/2}}$ versus the voltage, E. The results (*3*) showed the relation to be linear, and the slope yielded n-values within 1% of theory.

The evaluation of the theoretical relationship of current, time, and concentration (Equation 2) is presented by Figures 8, 9, and 10, and by the data of Table III. Before the initial phase of the work and without the authors' knowledge, data for the proof of Equation 2 had been taken with fairly short transition times (1 to 10^{-3} second) using oscillographic techniques. Because longer transition times could be more accurately and easily measured, the authors' data were taken to show the validity of Equation 2 for transition times between 4 and 60 seconds. Figure 8 illustrates the effect of current upon the potential-time curves obtained in the ion-amalgam reduction of a given concentration of cadmium ion. Figures 9 and 10 illustrate the linearity between $\tau^{1/2}$ and concentration; according to the equation, such a plot should be a straight line with slope dependent upon electrolyzing current. This is seen to be the case, both in the ion-amalgam reduction of cadmium (Figure 9) and in the reversible oxidation of potassium ferrocyanide at the platinum electrode (Figure 10).

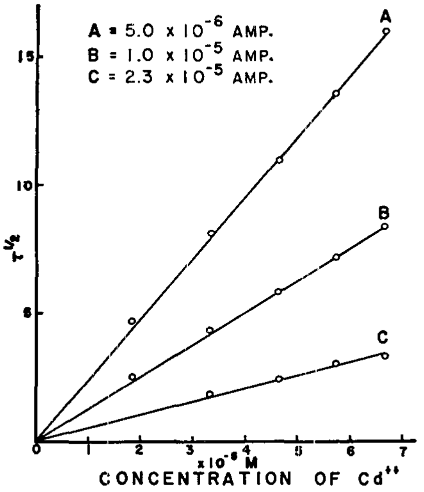

Figure 9. Relationship of concentration vs. $\tau^{1/2}$ at various current values

Mercury pool

Calculated values for the constants $\frac{I\tau^{1/2}}{C^\circ}$ and $\frac{I\tau^{1/2}}{nC^\circ D^{1/2}}$ from the experimental ion-amalgam reductions of lead, cadmium, and zinc, are summarized in Table III. From a quantitative standpoint, reproducibility in these values determines the applicability of the method. The average value of $\frac{I\tau^{1/2}}{nC^\circ D^{1/2}}$ for all ions studied had an average deviation of 4.3%. Considering the range of concentrations and currents employed, this deviation is not excessive and confirms the usefulness of Equation 2.

As a further check of the validity of Equation 2, the value of the area of the mercury surface was computed using this equation and the experimental average of $\frac{I\tau^{1/2}}{nC^\circ D^{1/2}}$. The computed result was 2.72 sq. cm. as compared with 2.76 sq. cm. calculated on the basis of the geometry of the pool. In practice, the effective area of the working electrode should be determined by running a chronopotentiogram on a standard solution and using Equation 2. Lead ions give well defined waves with platinum and mercury electrodes and stable lead solutions are easily prepared.

At the lower concentrations, graphical measurement of transition times is complicated by the greater slope in the upper regions of the curves, resulting from the increased relative value of the charging current component. Empirical calibration curves must then be constructed from data on standard solutions.

The precision obtained in graphical transition time determinations for mercury pool reductions is shown in Table I. The iodate data, contributed by Delahay and Mamantov (*3*), show precision superior to those obtained in this laboratory for cadmium ion. This would be anticipated from the increased dilution in the case of cadmium, and from the possibility of distortion due to the amalgamation of cadmium at the mercury surface. It is also probable that the cell design employed by Delahay and Mamantov gives a more constant mercury surface area.

Examination of Equation 1 reveals that when the scanning time, t, is equal to one fourth the total transition time, τ, the potential of the electrode becomes $E_{1/4}$, the "quarter-wave"

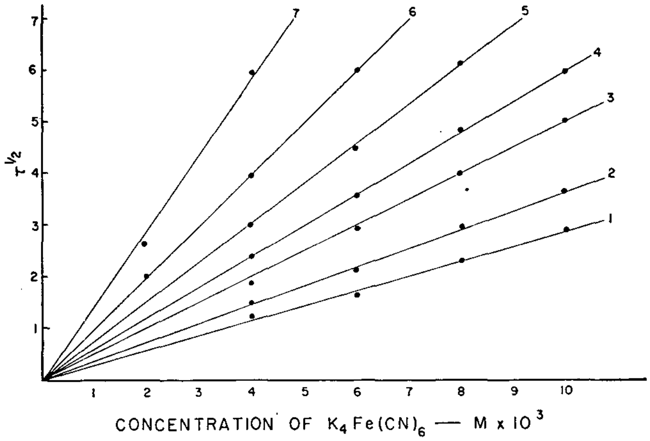

Figure 10. Relationship of concentration vs. $\tau^{1/2}$ at various current values

Anodic waves on platinum

1. 1.50×10^{-3} ampere
2. 1.19×10^{-3} ampere
3. 8.90×10^{-4} ampere
4. 7.47×10^{-4} ampere
5. 5.87×10^{-4} ampere
6. 4.40×10^{-4} ampere
7. 2.93×10^{-4} ampere

Table III. Chronopotentiometry of Lead, Cadmium, and Zinc Ions

(Mercury electrode. Effect of current-density and concentration)

Metal Ion	C, Moles/L. $\times 10^4$	I, Amp. $\times 10^3$	$I\tau^{1/2}$, Amp. Sec.$^{1/2}$ $\times 10^4$	$\frac{I\tau^{1/2a}}{C}$ $\times 10^{-3}$	$\frac{I\tau^{1/2a}}{nC\,D^{1/2}}$ $\times 10^{-5}$
Pb^{++}	10.0	0.273	13.9	1.39	2.22
	10.0	0.366	13.6	1.36	2.18
	10.0	0.180	14.1	1.41	2.25
	5.00	0.183	6.86	1.37	2.19
	5.00	0.136	7.02	1.40	2.24
	5.00	0.0898	6.97	1.39	2.22
	1.24	0.0328	1.89	1.53	2.44
	1.24	0.0258	1.81	1.46	2.34
	1.24	0.0263	1.82	1.47	2.35
Cd^{++}	6.90	0.136	8.82	1.28	2.38
	6.90	0.183	8.64	1.25	2.33
	6.90	0.230	8.38	1.22	2.28
	6.90	0.180	8.40	1.22	2.28
	3.45	0.0730	4.13	1.20	2.24
	3.45	0.0898	4.37	1.27	2.36
	3.45	0.0136	4.05	1.17	2.18
	3.45	0.0565	4.48	1.30	2.42
	1.00	0.0328	1.37	1.37	2.56
	1.00	0.0258	1.35	1.35	2.52
	1.00	0.0263	1.36	1.36	2.54
Zn^{++}	11.5	0.462	13.3	1.16	2.17
	11.5	0.366	13.7	1.19	2.22
	11.5	0.273	13.7	1.19	2.22
	5.75	0.136	7.00	1.22	2.28
	5.75	0.183	6.95	1.21	2.26
	5.75	0.0898	7.08	1.23	2.30
	1.15	0.0263	1.56	1.35	2.52
	1.15	0.0410	1.53	1.33	2.48
	1.15	0.0565	1.46	1.26	2.35
			Average		2.32
			Average deviation		0.10
			% average deviation		4.3%

[a] C in moles/ml.

potential. Quarter-wave potentials are characteristics of the ion itself, independent of its concentration, and are comparable to polarographic half-wave potentials. In the case of mixtures, the scanning time corresponding to the polarographic half-wave potential varies between $^1/_4\tau$ (in the case of no preceding waves) and $^1/_2\tau$ (in the case of larger preceding waves). The reproducibility of quarter-wave potentials was examined in replicate runs on lead-cadmium mixtures. The data in Table V (columns 8 and 9) show average deviations of 1 and 2 mv. for cadmium and lead, respectively. For the purpose of qualitative identification of ions in mixtures by chronopotentiometric means, this precision seems encouraging.

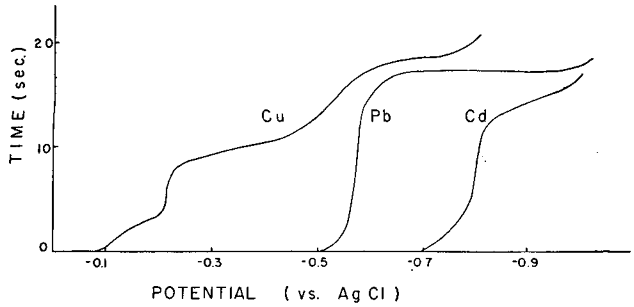

Figure 11. Potential-time curves for various metallic ions

Platinum electrode
Supporting electrolyte 0.1M KCl
Current 2.70 × 10^{-4} ampere
Copper, lead, and cadmium ions each 1 × 10^{-3} M

Platinum Electrode. While the cathodic working range of platinum is limited by the smaller hydrogen overvoltage, the electrode is advantageous in the respect that plated metals may be anodically stripped back into solution, and the solution reused for a subsequent run. Potential-time curves for the deposition of metal ions on platinum are illustrated in Figure 11. The curve for lead is as well defined and as reproducible as with the mercury electrode, but there has been a negative shift in the quarter-wave potential of about 0.15 volt. This shift is noted for all metal ion reductions, and is expected, as amalgam formation at the mercury electrode decreases the activity of the deposited metal. The upper portion of the curve for the cadmium ion is distorted by the simultaneous reduction of hydrogen ion. The case of the cupric ion (chloride media) is of particular interest; the curve shows three distinct potential holdups. The hydrogen ion reduction potential is altered by the presence of plated copper at the electrode.

Table IV. Chronopotentiometric Analysis of Anodic and Cathodic Waves

(Comparison of methods for obtaining transition times. Platinum electrode. Anodic oxidation of $10^{-2}M$ ferrous ion)

Transition Times by Recorder Method				Transition Times by Potential-Interval			
$E_{1/4}$ volt, *vs.* AgCl/Ag	τ, sec.	I, amp. × 10^4	$I\tau^{1/2}$, amp. sec.$^{1/2}$ × 10^3	Potential-interval, volt (*vs.* Ag/AgCl)	τ, sec.	I, amp. × 10^4	$I\tau^{1/2}$, amp. sec.$^{1/2}$ × 10^3
+0.440	2.78	14.5	2.42	+0.37 to +0.60	3.0	14.5	2.51
	4.39	11.5	2.41		4.7	11.5	2.49
	7.67	8.65	2.40		8.2	8.65	2.48
	10.5	7.15	2.31		11.6	7.15	2.43
	17.5	5.78	2.42		18.6	5.78	2.49
		Av.	2.39				2.48
	% average deviation		1.3				0.8
		Cathodic Reduction of Ferric Ion $10^{-2}M$					
+0.420	2.24	14.5	2.18	+0.37 to +0.60	2.6	14.5	2.34
	3.76	11.5	2.23		4.1	11.5	2.38
	7.02	8.65	2.29		7.40	8.65	2.35
	10.3	7.15	2.28		10.7	7.15	2.33
	16.2	5.78	2.33		16.7	5.78	2.36
		Av.	2.26				2.35
	% average deviation		2.0				0.6

Table V. Consecutive Reduction of Two Metal Ions

(Mixtures of lead and cadmium ions subjected to various polarizing currents. Mercury pool)

	1	2	3	4	5	6	7	8	9
Mixture Mole/L.	I, amp. × 10^3	P, amp. sec. × 10^4	$\frac{P}{C_1}$ × 10^{-3}	$\frac{P}{n_1C_1D_1^{1/2}}$ × 10^{-5}	Q × 10^4	$\frac{Q}{C_2}$ × 10^{-3}	$\frac{Q}{n_2C_2D_2^{1/2}}$ × 10^{-5}	E_1, volt (S.C.E.)	E_2, volt (S.C.E.)
Pb^{++}	0.0450	1.44	1.44	2.31	1.18	1.18	2.20	0.369	0.559
(10^{-4})	0.0410	1.48	1.48	2.36	1.24	1.24	2.31	0.368	0.557
+Cd^{++}	0.0565	1.53	1.53	2.44	1.14	1.14	2.12	0.366	0.560
(10^{-4})									
Pb^{++} (10^{-4})	0.0328	1.39	1.39	2.22	0.570	1.14	2.13	0.371	0.558
Cd^{++} (5 × 10^{-4})	0.0352	1.36	1.36	2.18	0.560	1.12	2.09	0.374	0.559
Pb^{++}	0.0410	1.40	1.40	2.24	2.54	1.27	2.38	0.366	0.558
(10^{-4})	0.0565	1.33	1.33	2.13	2.49	1.24	2.32	0.366	0.558
+Cd^{++}	0.0450	1.47	1.47	2.35	2.44	1.22	2.28	0.368	0.558
(2 × 10^{-4})	0.0730	1.50	1.50	1.39	2.46	1.23	2.30	0.366	0.562
Average			1.43	2.29		1.20	2.24	0.368	0.559
Average deviation			0.06	0.10		0.05	0.10	0.002	0.001
% average deviation			4.2	4.2		4.2	4.2		

$P = I\tau_1^{1/2}$ amp. sec.$^{1/2}$
$Q = I[(\tau_1 + \tau_2)^{1/2} - \tau_1^{1/2}]$ amp. sec.$^{1/2}$
C_1 and C_2 expressed as moles/ml.

Table VI. Consecutive Reduction of Three Metal Ions

(Various mixtures of lead, cadmium, and zinc ions subjected to various polarizing currents. Mercury pool.)

Mixture, Moles/L.	10 I, amp. $\times 10^3$	11 P $\times 10^4$	12 $\frac{P}{C_1}$ $\times 10^{-3}$	13 $\frac{P}{n_1C_1D_1^{1/2}}$ $\times 10^{-5}$	14 Q $\times 10^4$	15 $\frac{Q}{C_2}$ $\times 10^{-3}$	16 $\frac{Q}{n_2C_2D_2^{1/2}}$ $\times 10^{-5}$	17 R $\times 10^4$	18 $\frac{R}{C_3}$ $\times 10^{-3}$	19 $\frac{R}{n_3C_3D_3^{1/2}}$ $\times 10^{-5}$
Pb^{++} (10^{-4}) Cd^{++} (10^{-4})	0.0565	1.44	1.44	2.30	1.27	1.27	2.36	1.26	1.26	2.34
Zn^{++} (10^{-4})	0.0450	1.49	1.49	2.38	1.24	1.24	2.31	1.19	1.19	2.23
Pb^{++} (10^{-4}) Cd^{++} (5×10^{-5})	0.0565	1.50	1.50	2.40	0.616	1.23	2.30	0.645	1.29	2.40
Zn^{++} (5×10^{-5})	0.0410	1.49	1.49	2.38	0.619	1.24	2.31	0.627	1.25	2.34
Pb^{++} (5×10^{-5}) Cd^{++} (10^{-4})	0.0565	0.819	1.64[a]	2.62[a]	1.27	1.27	2.36	0.615	1.23	2.30
Zn^{++} (5×10^{-4})	0.0410	0.850	1.70[a]	2.72[a]	1.27	1.27	2.36	0.615	1.23	2.30
Average			1.48	2.36		1.25	2.34		1.24	2.32
Average deviation			0.02	0.04		0.02	0.03		0.02	0.03
% average deviation			1.4	1.7		1.6	1.3		1.6	1.3

[a] Not included in average (see text).

C values computed in moles/ml.
$P = I\tau_1^{1/2}$ amp. sec.$^{1/2}$, $Q = I[(\tau_1 + \tau_2)^{1/2} - \tau_1^{1/2}]$ amps. sec.$^{1/2}$
$R = I[(\tau_1 + \tau_2 + \tau_3)^{1/2} - (\tau_1 + \tau_2)^{1/2}]$ amp. sec.$^{1/2}$

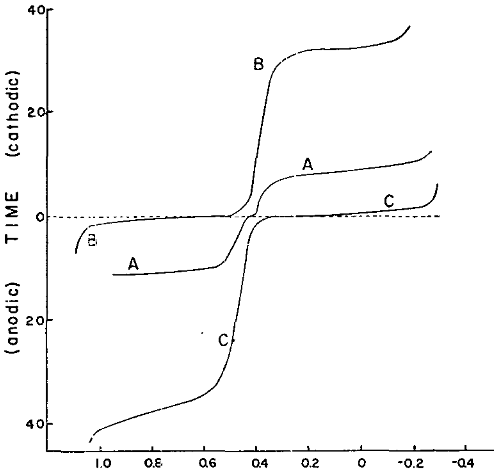

Figure 12. Potential-time curves for ferric-ferrous ions

Platinum electrode
Supporting electrolyte 0.5M HCl
Current 7.50 × 10^{-4} ampere
A. 0.005M $FeCl_2$ + 0.005M $FeCl_3$
B. 0.01M $FeCl_2$
C. 0.01M $FeCl_3$

The ferrous-ferric system is of interest because of its commercial importance and because it forms a convenient reversible redox system. Experiments were conducted on the anodic oxidation and cathodic reduction of these ions. Figure 12 shows the results of oxidizing a solution of ferrous ion, C, of reducing a solution of ferric ion, B, and of oxidizing and reducing an equal mixture of the two, A. Theoretical equations for the potential-time curves of this redox pair, A, show that the anodic-cathodic curve should cross the zero-time axis with finite slope and at the reversible potential of the electrode. Experiments show that this is not quite the case for the slope, and that a measurable degree of irreversibility is present. The degree of irreversibility was found to decrease with smaller current densities. This effect was shown to be independent of any IR drop in the solution or leads.

Consecutive Reductions. The final problem in this study was to investigate the qualitative and quantitative analysis of mixtures of cations by means of the chronopotentiometric method. When two or more ions are present in solution which are reduced at different potentials, the concentration of the ion first reduced may be calculated from its transition time by means of Equation 2. This is not true of subsequently reduced ions, as the total electrolysis current now divides between the diffusion currents of the first and second ions. The state of concentration polarization with respect to the second ion is reached less rapidly, and the transition time is correspondingly enhanced.

Potential-time curves for the reduction of three-component mixtures of lead, cadmium, and zinc are illustrated in Figure 13. The solution is 1 × $10^{-4}M$ with respect to each of its com-

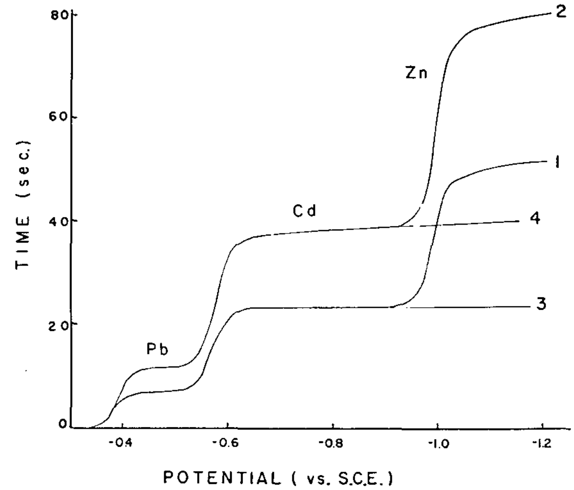

Figure 13. Chronopotentiometric curves for mixtures of lead, cadmium, and zinc ions in 0.1M potassium nitrate

Mercury pool
Concentration of each ion 1 × $10^{-4}M$
1. 5.65 × 10^{-5} ampere (Pb Cd, Zn)
2. 4.50 × 10^{-5} ampere (Pb Cd, Zn)
3. 5.65 × 10^{-5} ampere (Pb Cd)
4. 4.50 × 10^{-5} ampere (Pb Cd)

ponents. The effect of enhancement is clearly seen in the increasing height of successive breaks in each trace. A general equation found applicable to all diffusion-controlled chronopotentiometric systems studied may be used to find the concentration of each ion in a mixture:

$$i_0[(\tau_1 + \tau_2 + \ldots \tau_n)^{1/2} - (\tau_1 + \tau_2 + \ldots \tau_{n-1})^{1/2}] = \frac{\pi^{1/2}\, n_n F D_n{}^{1/2}\, C_n^\circ}{2} \quad (4)$$

When applied to a two-component mixture, this general equation reduces to Equation 7 of Delahay and Mamantov (*3*). With proper substitution, it also reduces to Equation 2 or 8 of the same authors. For the analysis of the three-component mixture under consideration here, Equation 4 may be written:

$$\frac{i_0 \tau_1{}^{1/2}}{n_1 C_1{}^\circ\, D_1{}^{1/2}} = \frac{i_0[(\tau_1 + \tau_2)^{1/2} - \tau_1{}^{1/2}]}{n_2 C_2{}^\circ\, D_2{}^{1/2}} = \frac{i_0[(\tau_1 + \tau_2 + \tau_3)^{1/2} - (\tau_1 + \tau_2)^{1/2}]}{n_3 C_3{}^\circ\, D_3{}^{1/2}} \quad (5)$$

The graphical transition times from the reduction of various mixtures of lead, cadmium, and zinc were used to calculate the data of Tables V and VI, by means of Equation 5. Reference to columns 4 and 7 (Table V) and 13, 16, and 19 (Table VI) indicates fair agreement in values for the fixed concentration of one component, under conditions of varying the concentration of the other components of a mixture. Only one set of values in Table VI shows appreciable errors: The discrepancy in the last two values of the column 13 which is to be expected in view of the small magnitude of the transition times encountered.

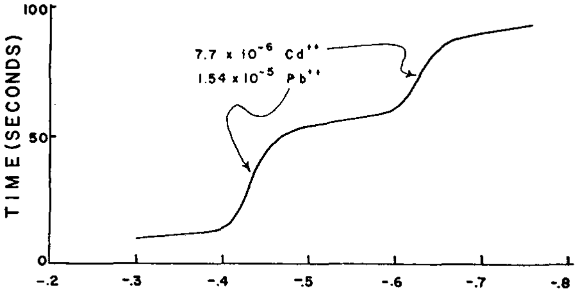

Figure 14. Potential-time curve for consecutive reductions of two ions at extreme dilution

Mercury pool

For analytical purposes, best results are obtained in mixture analysis by using smaller currents for the analysis of the more easily reduced species, so that longer transition times can be obtained and measured.

Inspection of Equation 4 shows that the analysis of a given wave does not require a knowledge of the concentrations or kinds of each species reduced at earlier potentials, but rather the experimental value of the square root of the total time elapsed between the initiation of electrolysis and the commencement of the wave in question. Figure 14 shows the degree of separation of two ions in an extremely dilute mixture. The waves are fairly well defined and transition times can be easily measured.

A consequence of the enhancement principle is the possibility of increasing the "detectability" of one ion by the addition of another which is more easily reduced. For instance, the response due to a cadmium concentration, too small to produce an accurately measurable wave, may be increased by the deliberate addition of a quantity of lead ion to the solution. This is illustrated in a slightly different manner by Figure 15. If it is assumed, for example, that a given concentration of cadmium ion alone yields a transition time of unity, as portions of the enchancing lead ion are added, the transition time for the added ion will increase as the square of its concentration in solution, shown by *A* in Figure 15. The enhancing effect upon the transition time of the cadmium ion initially present is shown by *B*. It may be shown from Equation 4 that the enhancement of the transition time is directly proportional to the concentration of the added ion. For the general case, the enhancement varies directly as the square root of the sum of all preceeding transition times. An upper useful limit of the procedure is reached when the concentration of the added ion is two to four times that of the ion initially present.

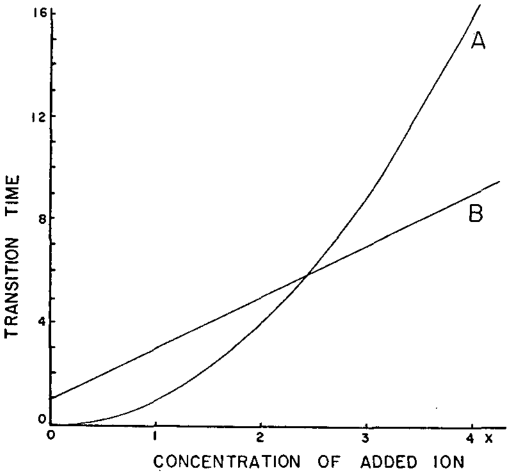

Figure 15. Enhancement of transition time of one ion by addition of more easily reduced ion

A. Transition time of added ion
B. Transition time of original ion

Potential-Interval Timing. In the absence of a recorder, it is possible to obtain satisfactory transition time measurements by timing the interval between two predetermined potentials, as observed on a pH meter. The recorder and interval-timing methods are compared in Table IV for the oxidation of ferrous ion and for the reduction of ferric ion at the platinum electrode. The precision of $I\tau^{1/2}$ values obtained by potential-interval timing actually exceeds that of the graphical method, but observed times are invariably somewhat longer. It is projected that an electronic relay be designed which would start and stop a timer at the two predetermined potentials, thereby making the interval-timing method automatic.

ACKNOWLEDGMENT

The authors gratefully acknowledge the aid of the Research Corp. for support of this project.

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RECEIVED for review May 24, 1954. Accepted January 31, 1955. Presented before the Division of Analytical Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y., 1954.

Spectrophotometric Determination of Niobium in Niobium-Bearing Steels

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A simple procedure for the colorimetric determination of niobium in niobium-bearing steels has been developed which involves only two separations: a perchloric acid hydrolytic precipitation and a chloroform extraction of the metal derivative of 8-quinolinol. The great bulk of the steel components is removed as soluble perchlorates in the first separation. Elements that may contaminate the niobic acid precipitate do not interfere because they do not react with 8-quinolinol, their oxinates (quinolinates) are insoluble in chloroform, or they are not extracted by chloroform from the ammoniacal citrate solution. The method makes use of the yellow color of chloroform solutions of niobium oxinate which shows maximum absorbance at 385 mμ. The method has been applied successfully to a niobium-bearing steel standard and to a series of composite steels. It involves a considerable saving in time and fewer mechanical operations without a sacrifice in accuracy.

The hitherto little known element, niobium, is no longer a laboratory curiosity but is an important element of metallurgy because of its role in preventing intergranular corrosion in stainless steels used at high temperatures.

Niobium is usually found along with tantalum, and their accurate separation by conventional wet methods is one of the most difficult of analytical problems. Schoeller (*9*) thoroughly studied the chemistry of these elements and developed the basic techniques for their separation and analysis. Modifications developed by Slavin (*10*) reduced the time for an analysis from about 15 to 5 days.

A number of new methods have recently been developed for the analytical separation of and the spectrophotometric determination of this element. Hiskey and coworkers (*2*) proposed the removal of interfering elements by the distillation of chlorinated products. Geld and Carroll (*6*) discussed a colorimetric method based on the yellow color of perniobic acid in concentrated sulfuric acid solution. Telep and Boltz (*11*) modified the method by making use of the absorbance of perniobic acid in the ultraviolet region. The yellow color of the niobium thiocyanate complex was also employed in a spectrophotometric method described by Freund and Levitt (*5*). Hiskey (*8*) also has developed a simultaneous spectrophotometric method for the determination of tantalum and titanium using the peroxy complexes in concentrated sulfuric acid solution.

In this paper is described a procedure involving the spectrophotometric measurement of a chloroform solution of niobium oxinate, which has been extracted from an ammoniacal citrate solution. By this procedure four to six determinations can be performed in a day. The precision and accuracy of the method are good.

REAGENTS AND EQUIPMENT

Ammonium hydroxide, 1 ± 0.01*N*, prepared from reagent grade.
Chloroform, reagent grade.
Citric acid solution, 25 ± 0.05 grams per 100 ml., prepared from reagent grade.
8-Quinolinol (oxime), analytical reagent.
Hydrochloric (37%) and nitric (70%) acids, 1 to 1 ratio, reagent grades.
Hydrochloric acid wash solution, 2%.
Niobium metal and oxide, C.P.
Oxine solution, 1 ± 0.01%, in chloroform.
Perchloric acid (71%), reagent grade.
Potassium bisulfate, analytical reagent.
Sulfuric acid (97%), reagent grade.
Sulfurous acid (freshly prepared).
Tantalum metal.
Beckman pH meter, Model H-2.
Beckman spectrophotometer, Model DU; 1.000-cm. matched cells; slit, 0.10 mm.

PROPOSED PROCEDURE

Weigh a 200-mg. sample (for steels containing between 0.3 and 3% niobium, otherwise change accordingly), place in a 600-ml. beaker, and dissolve with 50 ml. of a 1 to 1 mixture of 37% hydrochloric and 70% nitric acids. Carry along a reagent blank treated exactly like the sample in this and succeeding operations. Add 35 ml. of 71% perchloric acid and heat until the acid begins to boil. Cover the beaker and heat so that the perchloric acid boils under reflux for about 30 minutes (*6*). Cool, dissolve with 50 ml. of distilled water, add 50 ml. of a freshly prepared saturated sulfurous acid solution and a small quantity of paper pulp, and dilute to about 300 ml. with distilled water. Stir and boil gently for about 10 minutes, then digest for 2 hours. Cool, filter through No. 40 Whatman filter paper, wash well with 2% hydrochloric acid solution, and ignite the precipitate.

Fuse the oxides with 2.5 grams of potassium bisulfate in platinum crucibles at as low a temperature as possible in order to avoid spattering, then cool and moisten with 5 drops of concentrated 97% sulfuric acid. Repeat the fusion and, after cooling, dissolve the melt in about 40 ml. of a hot citric acid solution containing 10 ± 0.02 grams of the acid, and dilute to 250 ml. with distilled water in a volumetric flask. Transfer a 15-ml. aliquot to a 125-ml. separatory funnel and add 15 ml. of a recently standardized 1 ± 0.01*N* ammonium hydroxide solution. Mix thoroughly and then add 10 ml. of a 1 ± 0.01% solution of oxine in chloroform. Shake uniformly for 3.0 minutes, draw off the chloroform layer, and read the absorbance at 385 mμ. With the use of a previously calibrated working curve, calculate the percentage of niobium in the sample.

DEVELOPMENT OF PROCEDURE

Steel Composition. Niobium is one of approximately 25 elements which may be present in steels. These elements may be classified into four general groups: elements common to all steels such as iron, carbon, phosphorus, and sulfur; common alloying and scavenging elements such as chromium, tungsten, molybdenum, cobalt, vanadium, aluminum, and titanium; elements used in special steels such as niobium, tantalum, and zirconium; and elements generally occurring as impurities such as tin, antimony, and arsenic.

Perchloric Acid Digestion and Separation. Because of the large number of elements and the rather low selectivity of oxine, a prior separation of most of the elements in steel is necessary. The perchloric acid hydrolysis was selected because most of its salts are highly soluble and because of its strong oxidizing properties. The soluble perchlorates may be separated from the elements silicon, niobium, tantalum, tungsten, tin, and antimony, which form insoluble acids. Niobium and tantalum are precipitated quantitatively as the earth acids if the digestion is assisted with sulfurous acid (*1*).

The insoluble acids may be contaminated with molybdenum,

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vanadium, manganese, boron, and bismuth, which may be coprecipitated to a small extent if they are present in large amounts or if the precipitates are voluminous. Titanium and zirconium phosphate may contaminate the acid precipitate if appreciable amounts are present, but are dissolved in a prolonged digestion with perchloric acid. A more nearly pure precipitate can be obtained by keeping the acid concentration quite high, thus preventing the formation of basic salts (*12*).

Of the approximately 25 elements found in steels only six elements—namely, niobium, tantalum, tungsten, silicon, tin, and antimony—are precipitated by the perchloric acid hydrolysis. Molybdenum, manganese, vanadium, and boron may coprecipitate under special conditions. Titanium and zirconium may be present, as their phosphates are very slowly soluble in perchloric acid.

8-Quinolinol. The reactions of numerous organic complexing agents with niobium and tantalum solutions were examined. Of these 8-quinolinol appeared to be the most useful in aqueous tartaric and citric acid solutions. Niobium produced a yellow color, while tantalum produced no color. The color in the citric acid solution was much more intense than that in the tartaric acid solution.

The action of 8-quinolinol with the interfering elements that may remain from the acid hydrolysis is summarized in Table I. Many of the data were obtained from Feigl (*4*); however, the solubilities were also verified in this work. Boron, phosphorus, and silicon do not react. The reaction of tin and antimony are doubtful, since the extract does not show any absorbance. Molybdenum and tungsten form insoluble oxinates in chloroform. This is important, because tungsten is completely precipitated by the perchloric acid digestion and molybdenum, which may be coprecipitated, is often found in relatively large quantities. Tantalum and vanadium salts also are not extracted. Although the titanium oxinate is extracted and has high absorbance values at 385 mμ, it did not introduce an error in composite steel samples (Table II), and thus it must have been eliminated as a soluble perchlorate in the initial separation. Titanium probably exists chiefly as the carbide (*3*) and appears to go into solution quite readily.

Table I. Action of Oxine on Possible Interfering Elements

Element	Action	Solubility of Oxinate in $CHCl_3$	Extraction from Ammoniacal Citrate Solution, pH 9.4
Antimony	Doubtful	...	Not extracted
Boron	No action	...	...
Manganese	Reacts	Soluble	Extracted
Molybdenum	Reacts	Insoluble	Not extracted
Niobium	Reacts	Soluble	Extracted
Phosphorus	No reaction	...	...
Silicon	No reaction	...	...
Tantalum	Reacts	Soluble	Not extracted
Tin	Doubtful	...	Not extracted
Titanium	Reacts	Soluble	Extracted
Tungsten	Reacts	Insoluble	Not extracted
Vanadium	Reacts	Soluble	Not extracted
Zirconium	Reacts	Soluble	Extracted

Thus, of the potential interfering elements only manganese and zirconium remain to be considered. Manganese may be present because of coprecipitation and zirconium possibly because of the slow attack by perchloric acid. The procedure calls for an extended digestion in order for the perchloric acid to act on any resistive compounds of zirconium that may be present. The results obtained with synthetic samples (Table II) containing relatively high ratios of manganese and zirconium to niobium indicated no interference.

Table II. Analyses of Composite Samples Prepared from NBS Samples[a]

Sample	Niobium, % In sample	Niobium, % Found	Error, %	Impurity Ratio
1	0.40	0.39	−2.5	Si 5:1
2	0.45	0.46	+2.2	Si 5:1
3	0.58	0.57	−1.7	Mo 1:1
4	0.31	0.31	0.0	Ti 1:1, Mn 3:1
5	0.115	0.116	+0.37	P 1:1, Mn 9:1
6	0.105	0.103	−1.9	V 1:2, Si 6:1
7	0.105	0.103	−1.9	Mo 1:1, Mn 6:1
8	0.72	0.72	0.0	W 1:1
9	0.73	0.72	−1.4	Zr 1:1
10	0.74	0.72	−2.7	Ta 1:1
11	0.198	0.196	−1.0	V 1:1, Mn 3:1
12	0.74	0.75	+1.4	Sn 1:1
13	0.74	0.73	−1.4	Sb 1:1
14	0.67	0.66	−1.5	B 1:1
15	0.248	0.243	−2.0	Ti 1:1, Si 2:1
16	0.102	0.102	0.0	W 5:1, Mn 10:1
17	0.72	0.73	+1.4	Zr 1:1
18	0.74	0.73	−1.4	Ta 1:1

[a] Tantalum, zirconium, antimony, and boron added to NBS samples.

Optimum Conditions for Extraction. To determine the proper conditions for the complexing reaction and extraction, 15-ml. aliquots of 4% citric acid solution containing 0.080 mg. of niobium and 0.100 mg. of tantalum were treated with different quantities of 1*N* sodium and ammonium hydroxide solutions. These were then diluted to 35 ml. with water and extracted with 10 ml. of a 1% solution of oxine in chloroform.

The effects that various volumes of the 1.0*N* ammonium and sodium hydroxide solutions have on the amount of niobium oxinate and tantalum oxinate extracted is shown in Figure 1. The absorbance was measured at 385 mμ.

The solution is highly buffered at about a pH of 9.4 when 12 to 18 ml. of 1.0*N* ammonium hydroxide are added to 15 ml. of a 4% citric acid solution of the sample.

Under these conditions the niobium oxinate extract exhibits a well defined color while the tantalum oxinate shows no absorbance and so does not interfere.

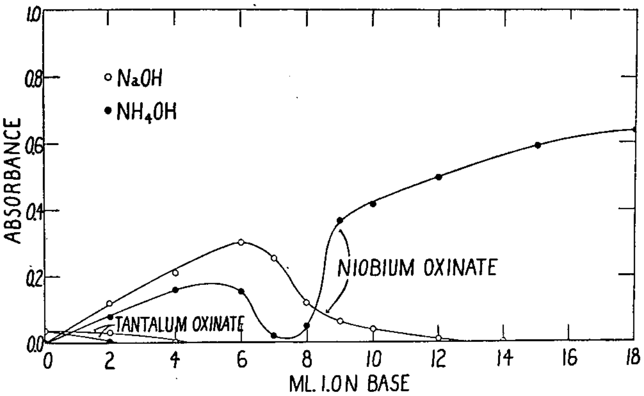

Figure 1. Effect of bases on chloroform extraction of niobium and tantalum oxinates

Effect of Time and Light. Chloroform solutions of certain other 8-quinolinates have been reported to undergo slow photochemical decomposition (*7*), especially when exposed to bright light. A niobium sample, along with a blank, was extracted under the described conditions and the persistence of the developed color was studied for a period of 30 hours. The samples were kept in the Beckman Corex cells and protected constantly from light. The effect of the elapsed time was found to be negligible (Table III), as the difference between the absorbance value of the oxinate and the blank remained constant. However, samples exposed to strong sunlight decomposed rapidly.

Precision. The precision of the method, based on six samples of niobium containing 0.040 mg. per sample, is shown in Table IV. The deviations of the readings are within the experimental error.

Absorption Characteristics. The absorbance of niobium oxinate shows a maxima at 385 mμ, Figure 2. The strong absorption band below 360 mμ is due to the unreacted oxine; therefore its concentration must be kept as low as possible. One 10-ml. portion of 1% solution was found to be sufficient to react with all the niobium present in the samples used in this work.

Seven samples of niobium oxinate–chloroform solution extracted from the buffered citric acid solution were studied. At the concentrations employed the interference of the unreacted oxine was shown to be negligible, Figure 2. Also there is no displacement of the band as the concentration of niobium is changed.

The absorptivity *a* (the relation $\log I_0/I = acl$) averaged 122 when the concentration, *c*, was expressed in grams of niobium per liter and the light path was 1 cm. The calculated molar absorptivity, ϵ, averaged 1.13×10^4.

The absorbance data at 385 mμ adhere to Beer's law. A graph of the data at 385 mμ from Figure 2 *vs.* concentration of niobium shows a straight line intercepting at the origin. Thus, such factors as ionization, dissociation, and association may be considered to be absent. Since these factors are negligible, they can have no effect upon the distribution constant between the two liquid phases. In the procedure, accurate control of the volumes of the two immiscible liquids is possible and recommended. Also, the oxinate is highly soluble in the chloroform solution. Therefore, under these conditions only one extraction is necessary. Results obtained using three extractions were no better than those obtained with one extraction.

In terms of milligrams of niobium, the concentration range corresponds to about 0.02 to 0.08 mg. per 15-ml. sample or from about 1.5 to 6.0 p.p.m. The niobium concentrations were brought into this optimum range by the proper selection of sample size and/or aliquoting.

ANALYSIS OF STEEL SAMPLES

The validity of the procedure was checked by the use of NBS niobium-bearing steel 123a, which contains tantalum and titanium in small quantities. The results are summarized in Table V. The average error of the seven samples is 1.3% based upon the amount of niobium present.

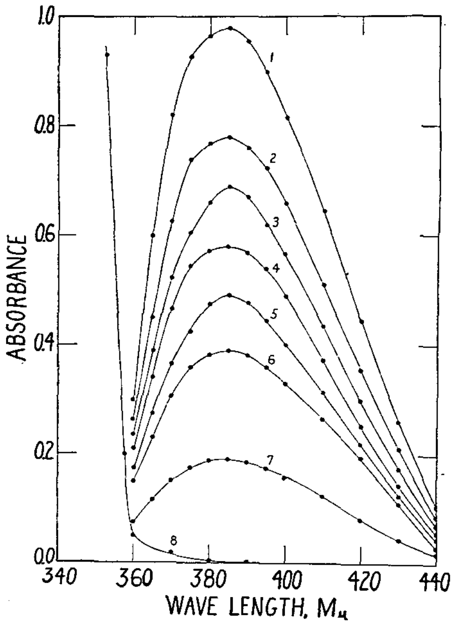

Figure 2. Absorption spectra of niobium oxinate

Present per 15 ml. of solution

1. 0.120 mg. of niobium
2. 0.096 mg. of niobium
3. 0.084 mg. of niobium
4. 0.072 mg. of niobium
5. 0.060 mg. of niobium
6. 0.048 mg. of niobium
7. 0.024 mg. of niobium
8. 200 mg. of oxine

Table III. Effect of Time on Color Stability of Niobium Oxinate in Chloroform (Protected from Light)[a]

Time, Hours	Absorbance Total	Absorbance Blank	Absorbance Nb oxinate
0	0.442	0.125	0.317
1	0.442	0.126	0.316
3	0.443	0.129	0.315
6	0.447	0.130	0.317
18	0.450	0.132	0.318
24	0.452	0.134	0.318
30	0.453	0.135	0.318

[a] Sample contained 0.040 mg. of niobium per 15 ml. of solution.

Table IV. Precision of Chloroform Extraction of Niobium Oxinate

Sample	Niobium, Mg. Present	Niobium, Mg. Found	Absolute Error, Mg.
1	0.040	0.039	−0.001
2	0.040	0.039	−0.001
3	0.040	0.038	−0.002
4	0.040	0.039	−0.001
5	0.040	0.038	−0.002
6	0.040	0.039	−0.001
	Average	0.039	−0.001

Table V. Analysis of Niobium-Bearing Steel 123a

Sample	Niobium, % Certified	Niobium, % Found	Absolute Error, %
1	0.75	0.74	−0.01
2	0.75	0.74	−0.01
3	0.75	0.73	−0.02
4	0.75	0.75	0.00
5	0.75	0.76	+0.01
6	0.75	0.74	−0.01
7	0.75	0.75	0.00
	Average	0.74	0.01

As steel 123a contained only tantalum and titanium in small quantities, composite samples were prepared with steel 123a and other NBS samples to provide samples with relatively high ratios of the potentially interfering elements to the amount of niobium present. The results of these analyses are summarized in Table II including the ratio of the impurities. The elements that may occur as major constituents, such as silicon, manganese, and tungsten, were tested in different ratios. Less important elements were examined only once.

The errors of the determinations are small, although the ratios of the interfering elements to niobium are large. The procedure effectively removes the interfering elements although they are present in relatively large quantities.

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RECEIVED for review May 15, 1954. Accepted January 3, 1955. Submitted by Asdrubal Garcia-Porrata in partial fulfillment for the degree of doctor of philosophy. Presented before the Southeastern Regional Meeting of the AMERICAN CHEMICAL SOCIETY, Auburn, Ala., October 1952.

Spectrophotometric Determination of Aliphatic Sulfides in Crude Petroleum Oils and Their Chromatographic Fractions

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Research programs for the study of organic sulfur compounds in crude petroleum by chromatographic techniques have demanded the development of methods for the determination of the sulfur types. A method, based upon a modification of the procedure of Hastings, is described for the determination of aliphatic sulfides in small samples of high molecular weight material such as a crude oil chromatographic fraction. Increased sensitivity has permitted the use of small samples, which substantially reduces sample absorption in the ultraviolet region, thus making possible the analysis of samples containing high concentrations of aromatic hydrocarbons. Interference due to formation of hydrocarbon-iodine complexes has been shown to be negligible. This method should be useful for following the course of the separation of aliphatic sulfides from crude petroleum by chromatography or thermal diffusion. As the method is directly applicable to crude oil, it should be valuable in the characterization of crude petroleum prior to refinery operations.

IN THE course of studying sulfur compounds in crude oil, it has become necessary to develop methods for the determination of various sulfur types in crude oil and crude oil chromatographic fractions (*7*). Such methods must be applicable to very small samples of high average molecular weight which are complex in nature and, in many instances, dark in color.

The classical method developed by Ball (*2*) for the schematic determination of the sulfur types in petroleum distillates requires large samples (250 ml.). Crude petroleum contains sulfur compounds which exhibit such low reactivities toward the reagents used in the Ball procedure that only a low percentage of the total sulfur in most crudes can be accounted for. The method is also time-consuming.

Recently a unique method for the determination of aliphatic sulfides in petroleum distillates boiling below about 450° F. was developed by Hastings (*4*). This method, which is based upon the formation of intermolecular complexes of aliphatic sulfides and iodine having intense absorption at 308 mμ, cannot be applied to crude petroleum because of the intensity of the absorption due to the sample itself.

This paper describes a procedure, based upon a modification of Hastings' method, for the determination of sulfide sulfur in crude petroleum or crude petroleum chromatographic fractions. Since complex formation with iodine follows the equilibrium

$$\text{Aliphatic sulfide} + I_2 \rightleftarrows \text{aliphatic sulfide}\cdot I_2 \qquad (1)$$

consideration of the mass action law has led to the supposition that the sensitivity of the Hastings' procedure could be increased considerably by using much higher iodine concentrations. This increased sensitivity, together with the use of a Beckman DU spectrophotometer with photomultiplier attachment, made possible the analysis of crude oils through the use of lower sample concentrations which, in turn, decreased the sample background absorption. The rapid decrease in the absorbance of the iodine blend after being placed in the dark (cell compartment) has not been observed.

Hastings and Johnson (*5*) have recognized the mass action law effect and have proposed a similar modification using an iodine concentration slightly lower than that employed in the following procedure. Considering the differences in iodine concentration, solvent, and technique, the results by the two modified procedures agree remarkably well.

PROCEDURE

Apparatus. Beckman DU spectrophotometer equipped with the photomultiplier attachment and one pair of 1-cm., matched, fused-quartz cells.

Volumetric flasks and pipets.

Reagents and Chemicals. Carbon tetrachloride, technical.

Iodine, analytical reagent grade.

Iodine reagent, prepared by dissolving exactly 10.00 grams of iodine in carbon tetrachloride and diluting to 1 liter.

Procedure. A weighed sample is dissolved in carbon tetrachloride and diluted to the proper volume. Dilutions of this solution may be prepared in order to produce absorption of the sample-iodine blend falling in the range of optimum accuracy (an absorbance of between 0.4 and 1.0). For very small samples, such as chromatographic fractions, a micro- or semimicrobalance may be used to weigh the required quantity of sample for dilution to 25 ml. in order to conserve material. A list of appropriate sample weights is given below.

Aliphatic Sulfide Sulfur in Sample, %	Approximate Weight of Sample Diluted to 25 Ml. to Obtain Absorbance between 0.4 and 1.0, Mg.
0.05	60–140
0.10	30–70
0.20	15–35
0.40	8–18
0.60	5–12
0.80	4–9
1.0	3–7
1.5	2–5
2.0	1–3

Dilute 1 ml. of the iodine reagent to 10 ml. with the above sample solution at the proper concentration.

Dilute 1 ml. of carbon tetrachloride to 10 ml. with the same sample solution and use this solution as the reference solution for the spectrophotometric measurement.

Immediately after preparing the sample-iodine blend and the reference solution, obtain the absorbance of the iodine blend at 310 mμ. Make the measurements as soon as the solutions have been placed in the cell compartment. As the absorbance has been noticed to increase slowly in the case of crude oils if the iodine blend is allowed to stand, it is recommended that measurements consistently be made immediately after the preparation of the blend.

A blank value for the iodine reagent is obtained as follows: One milliliter of the iodine reagent is diluted to 10 ml. with carbon tetrachloride and the absorbance of this solution measured at 310 mμ using carbon tetrachloride as the reference.

Calculations. The weight percentage of aliphatic sulfide sulfur is calculated as follows:

$$\text{Wt. \% aliphatic sulfide sulfur} = \frac{(A_1 - A_2) \times 100}{400 \times 0.9 \times c}$$

where A_1 is the absorbance of the sample-iodine blend at 310 mμ and A_2 is the absorbance due to iodine (blank). Subtraction of this blank not only accounts for absorption due to iodine in the blend but also corrects for differences in cell absorbances, as pointed out by Hastings (*4*). The average absorptivity is taken to be 400 liters per gram-cm. The absorptivity for the sulfide-iodine complex is calculated on the basis of the total aliphatic sulfide concentration rather than on the true concentration of the complex. The factor 0.9 is the dilution factor necessary to correct for diluting 1 ml. of iodine to 10 ml. with the sample solution, the concentration of which in grams per liter is represented by c in the equation above.

Table I. Absorptivities of Iodine Complexes of Sulfur Compounds in Carbon Tetrachloride at an Iodine Concentration of 1 Gram per Liter

Compound	Source	Absorptivity, Liters/Gram-Cm.[a]
Thiacyclopentane	API, RP48A, Bur. Mines, Laramie, Wyo.	433 ± 4
2-Methylthiacyclopentane	API, RP48A, Bur. Mines, Laramie, Wyo.	403 ± 3
2-Thiapropane	API-USBM standard sample 13-5S	185 ± 1
2-Thiabutane	API-USBM standard sample 902-5S	265 ± 2
2-Thiapentane	API-USBM standard sample 18-5S	262 ± 1
3-Methyl-2-thiabutane	API-USBM standard sample 20-5S	308 ± 2
3-Thiapentane	API, RP48A, Bur. Mines, Laramie, Wyo.	364 ± 1
2,2,4,4-Tetramethyl-3-thiapentane	API, RP48A, Bur. Mines, Laramie, Wyo.	301 ± 1
Thiacyclobutane	API-USBM standard sample 10-5S	198 ± 1
Thiacyclohexane	API-USBM standard sample 17-5S	324 ± 1
5-Thianonane	Eastman Kodak Co.	379 ± 1
2,6-Dimethyl-4-thiaheptane	Eastman Kodak Co.	280 ± 2
6-Thiaundecane	Eastman Kodak Co.	385 ± 3
2,8-Dimethyl-5-thianonane	Eastman Kodak Co.	391 ± 2
7-Thiatridecane	Eastman Kodak Co.	403 ± 4
8-Thiapentadecane	Eastman Kodak Co.	402 ± 3
1,3-Diphenyl-2-thiapropane	Eastman Kodak Co.	72 ± 0
Diphenylthiamethane	Eastman Kodak Co.	1.5 ± 0.0
1-(*p*-Methylphenyl)-1-thiaethane	Eastman Kodak Co.	17 ± 0
1-(*p*-Methylphenyl)-1-thiapropane	Eastman Kodak Co.	32 ± 1
5,6-Dithiadecane	Eastman Kodak Co.	4.8 ± 0.1
1,2-Diphenyldithiaethane	Eastman Kodak Co.	0.3
1,4-Diphenyl-2,3-dithiabutane	Eastman Kodak Co.	2.5 ± 0.0
tert-Butyl trisulfide	Phillips Petroleum Co.	4.0 ± 0.2
tert-Butyl polysulfide	Phillips Petroleum Co.	6.9
1-Dodecanethiol	API, RP48A, Bur. Mines, Laramie, Wyo.	13.1 ± 0.2
1-Decanethiol	Matheson Coleman & Bell	11.6 ± 0.1
1-Tetradecanethiol	Matheson Coleman & Bell	9.9 ± 0.2
1-Hexadecanethiol	Matheson Coleman & Bell	9.0 ± 0.3
1-Octadecanethiol	Matheson Coleman & Bell	10.6 ± 0.3
1,1-Dimethyl-1-hexanethiol	Matheson Coleman & Bell	12.5 ± 0.0
p-Methylbenzenethiol	Eastman Kodak Co.	4.2 ± 0.5
2-Phenyl-1-ethanethiol	Matheson Coleman & Bell	12.2 ± 0.1
Phenylmethanethiol	Eastman Kodak Co.	5.3 ± 0.2
9,10-Dithia-9,10-dihydroanthracene	Eastman Kodak Co.	0.6
Dibenzothiophene	Eastman Kodak Co.	0.5
2,5-Di-*tert*-butylthiophene	Socony-Vacuum Oil Co., Inc.	0.6
2,5-Di-*tert*-octylthiophene	Socony-Vacuum Oil Co., Inc.	0.3

[a] Determined at 26° ± 1° C.

DISCUSSION OF METHOD

Wave Length of Maximum Absorption. The average wave length of maximum absorption for the aliphatic sulfide-iodine complexes at an iodine concentration of 1 gram per liter (in carbon tetrachloride) was found to be 310 mμ (Figure 1), in good agreement with the value given by Hastings for an iodine concentration of only 0.1 gram per liter in iso-octane (2,2,4-trimethylpentane).

Effect of Iodine Concentration. A standard solution of iodine in carbon tetrachloride was added from a buret to 95 ml. of a 0.000159*M* solution of thiacyclopentane (tetrahydrothiophene) in carbon tetrachloride. The absorbance at 310 mμ was measured after each increment of iodine solution was added. The small quantity of liquid required for the measurement was returned to the bulk of the solution. No correction was made for small changes in the concentration of the solution owing to evaporation of the solvent.

The results, shown in Figure 2, indicate that a large increase in sensitivity is obtained by increasing the concentration of iodine in the sulfide-iodine blend. An iodine concentration of 1 gram per liter in the sample-iodine blend was arbitrarily chosen as desirable for the method. Because of the greater solubility of iodine in carbon tetrachloride than in iso-octane carbon tetrachloride was chosen as the solvent for these investigations. Hildebrand and Scott (*6*) list the solubility of iodine in iso-octane and carbon tetrachloride as 0.592 and 1.147 mole %, respectively, which correspond to about 9 and 30 grams per liter.

An iodine concentration higher than 1 gram per liter would involve the use of iodine blanks which are objectionably high. The possibility of lowering the blank value has not been investigated, but it might be accomplished through purification of the carbon tetrachloride by passing it through a silica or alumina column. A higher iodine concentration than 1 gram per liter, even if the blank could be reduced, would not increase the absorptivity significantly.

Theoretical treatment of these data shows that the system follows the equilibrium formation of a 1 to 1 complex between the sulfide and iodine.

Absorptivity of the Aliphatic Sulfide-Iodine Complex. The absorptivities of the iodine complexes of a number of sulfur compounds were determined at an iodine concentration of 1 gram per liter. These values are given in Table I.

The absorptivities of the sulfide-iodine complexes are also plotted in Figure 3. Values for the symmetrical di-*n*-alkyl sulfides (except 2-thiapropane) fall on a straight line as shown. As is observed so often in physical data of a homologous series of compounds, the compound containing the methyl group (or groups) is out of line. In general, it is also observed that absorptivities for the sulfides with *sec*- or *tert*-alkyl groups are somewhat low. The five-membered cyclic sulfides have slightly higher absorptivities than the acyclic sulfides.

As it is unlikely that sulfides of low molecular weight, such as 2-thiapropane and 2-thiabutane, are to be found to any great extent in crude oil, and cyclic sulfides such as thiacyclopentane derivatives are thought to be present in relatively high concentration, it is concluded that an average absorptivity of 400 liters per gram-cm. should be used for the determination of sulfides in crude oil and crude oil chromatographic fractions. This value, which is based on limited data, must suffice until more work is done on the separation and identification of sulfides in crude oil by techniques such as chromatography or thermal diffusion where no extensive thermal degradation takes place.

Table I shows that the sulfur types other than the aliphatic sulfides form iodine complexes with absorptivities which are only

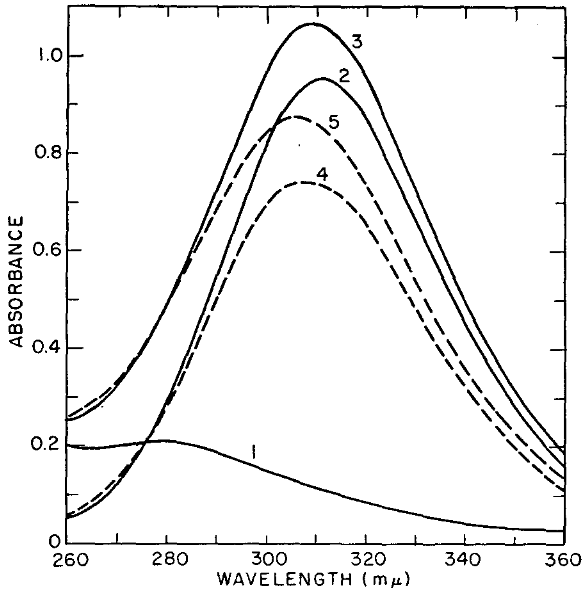

Figure 1. Absorption spectra of typical aliphatic sulfide-iodine blends in carbon tetrachloride

1. Iodine blank (1 gram per liter)
2. Curve 3 minus curve 1
3. 2-Methylthiacyclopentane-iodine blend
4. Curve 5 minus curve 1
5. 7-Thiatridecane-iodine blend

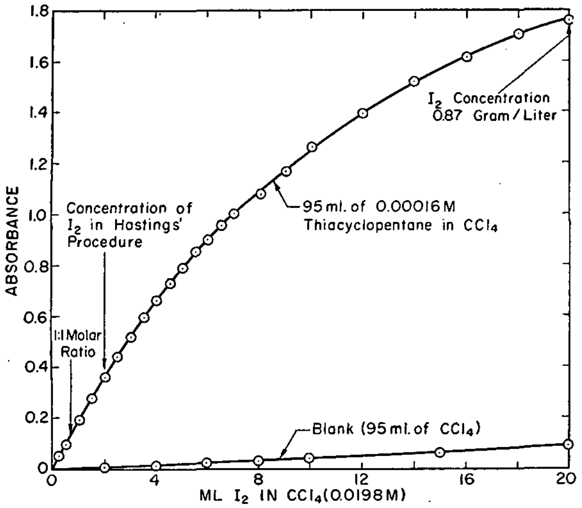

Figure 2. Effect of iodine concentration upon absorption at 310 mμ due to complex formation between thiacyclopentane and iodine

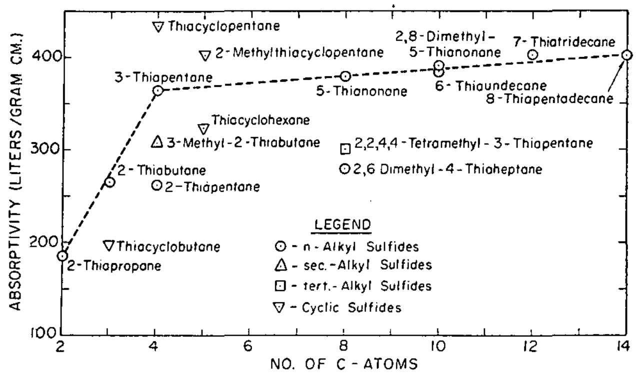

Figure 3. Absorptivities of some aliphatic sulfide-iodine complexes in carbon tetrachloride at an iodine concentration of 1 gram per liter

Dotted line drawn through values for di-*n*-alkyl sulfides

very small percentages of the average absorptivities for aliphatic sulfides.

Nitrogen- and oxygen-containing compounds which would also form complexes with iodine are expected to be present in only relatively small concentration and would not cause serious interferences.

Interference Owing to Formation of Aromatic Hydrocarbon-Iodine Complexes. Even though small absorptivities were reported by Hastings for the aromatic hydrocarbons, it was feared that crude oils and especially the latter fractions from a chromatographic fractionation would contain such high concentrations of aromatics in proportion to the aliphatic sulfides that interference because of the formation of aromatic-iodine complexes would be serious.

Andrews and Keefer (*1*) have determined the equilibrium constants and molar absorptivities of a number of aromatic-iodine complexes. These values, along with the wave lengths of maximum absorption, are given in Table II.

The equilibrium constants and molar absorptivities for iodine complexes of several additional hydrocarbons readily available in pure form have been determined. The method used by Andrews and Keefer and by Blake, Winston, and Patterson (*3*) was followed. This method involves the following easily derived equation:

$$\frac{l[I_2]}{A} = \frac{1}{K_c\epsilon_c} \times \frac{1}{[\mathrm{Ar}]} + \frac{1}{\epsilon_c} \text{ for } [\mathrm{Ar}] >> [\mathrm{I_2}] \qquad (2)$$

where $[I_2]$ and [Ar] are the molar concentrations of iodine and aromatic hydrocarbon, respectively, l is the cell path length, A is the absorbance at the wave length of maximum absorption, K_c is the equilibrium constant, and ϵ_c is the molar absorptivity of the complex. Five or more points were obtained at different aromatic and iodine concentrations, and the values for ϵ_c and K_c for Equation 2 were obtained by the method of least squares. A typical plot of values for the ethylbenzene-iodine complex is

Table II. Equilibrium Constants for Some Aromatic Hydrocarbon-Iodine Complexes in Carbon Tetrachloride at 25° C.[a]

Aromatic	λ_{max}, mμ	$\epsilon_{max} \times 10^{-3}$	K_c
Benzene	292	16.4	0.15
Toluene	302	16.7	0.16
o-Xylene	316	12.5	0.27
m-Xylene	318	12.5	0.31
p-Xylene	304	10.1	0.31
Mesitylene	332	8.85	0.82
Durene	332	9.00	0.63
Pentamethylbenzene	357	9.26	0.88
Hexamethylbenzene	375	8.20	1.35
Hexaethylbenzene	378	16.7	0.13
Bromobenzene	290	10.4	0.13
Phenanthrene	396	13.0	0.15
Naphthalene	360	7.64	0.25
Styrene	330	7.35	0.31
Stilbene	373	7.14	0.31
Bibenzyl	304	11.2	0.46

[a] Determined by Andrews and Keefer (*1*).

Table III. Equilibrium Constants for Some Aromatic Hydrocarbon-Iodine Complexes in Carbon Tetrachloride at 26° C.[a]

Aromatic	λ_{max}, mμ	$\epsilon_{max} \times 10^{-3}$	K_c
Ethylbenzene	303	12.1	0.22
p-Di-*tert*-butylbenzene	308	9.9	0.30
Naphthalene	360	6.2	0.28
Acenaphthene	399	5.7	0.43

[a] Determined by authors.

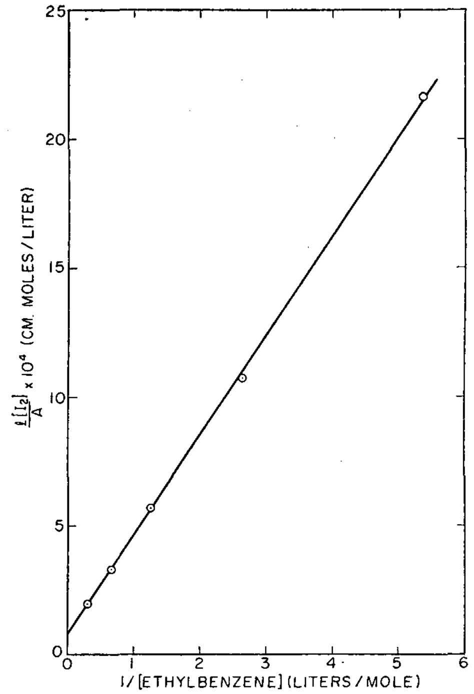

Figure 4. Plot of equilibrium data for ethylbenzene-iodine complex

presented in Figure 4. The results of these measurements are given in Table III.

On the basis of values in the above tables, it is estimated that ϵ_c (for λ_{max}) is about 15,000 liters per mole cm. and K_c is about 1.0 liter per mole for the aromatic hydrocarbon-iodine complexes. All assumptions or approximations here were chosen so as to maximize the interference—i.e., the average values for ϵ_c and K_c were chosen to be higher than they probably are for most samples which will be encountered.

Using these values, and assuming that the entire sample consists of aromatic hydrocarbons, the interference, in terms of apparent weight per cent aliphatic sulfide sulfur can be calculated as follows: For the equilibrium

$$\text{Aromatic} + I_2 \rightleftarrows \text{aromatic}\cdot I_2, \tag{3}$$

the equilibrium constant is written

$$K_c = \frac{[\text{aromatic}\cdot I_2]}{[\text{aromatic}]\ [I_2]} \tag{4}$$

Thus,

$$[\text{Aromatic}\cdot I_2] = K_c\ [\text{aromatic}]\ [I_2] \tag{5}$$

However,

$$A = \epsilon lc \tag{6}$$

where A is the absorbance, ϵ is the molar absorptivity of the aromatic-iodine complex, in liters per mole centimeter, l is the cell path length in centimeters, and c is the concentration of the complex in moles per liter. Substituting Equation 5 in Equation 6,

$$A = \epsilon l K_c\ [\text{aromatic}]\ [I_2] \tag{7}$$

But

$$\text{Apparent wt. \% aliphatic sulfide sulfur} = \frac{A \times 100}{l \times \text{grams of sample/liter} \times a} \tag{8}$$

where a is the average absorptivity of aliphatic sulfide-iodine complexes in liters per gram (of aliphatic sulfide sulfur) cm. Substituting Equation 7 in Equation 8,

$$\text{Apparent wt. \% aliphatic sulfide sulfur} = \frac{\epsilon l K_c\ [\text{aromatic}]\ [I_2] \times 100}{l \times \text{grams of sample/liter} \times a} \tag{9}$$

Since it was assumed, for simplicity, that

$$[\text{aromatic}] = \frac{\text{grams of sample/liter}}{\text{mol. wt.}} \tag{10}$$

Equation 9 becomes

$$\text{Apparent wt. \% aliphatic sulfide sulfur} = \frac{\epsilon K_c\ [I_2] \times 100}{\text{mol. wt.} \times a} \tag{11}$$

Substituting the values:

ϵ = 15,000 liters per mole cm.
K_c = 1.0 liters per mole
$[I_2]$ = 0.00394 mole per liter
a = 400 liters per gram-cm.
mol. wt. = 300 grams per mole

the apparent weight % aliphatic sulfide sulfur = 0.049 for a sample of average molecular weight 300. For samples of average molecular weight 200, 400, and 500 it becomes 0.074, 0.037, and 0.030, respectively. These data represent greater than maximum interference which would be encountered in any actual sample of crude oil. It was presumed that actual interference would be nearer 0.01 to 0.02 weight % aliphatic sulfide sulfur (absolute).

A sample of crude oil low in sulfur and high in aromatics was obtained from H. M. Smith (U. S. Bureau of Mines, Petroleum Experiment Station, Bartlesville, Okla.) which was reported to contain 0.12% by weight total sulfur for the whole crude and 30 to 32% by volume aromatics in the 300° to 600° F. boiling range fractions. This was considered a representative sample relative to aromatic content, and was assumed for the purpose of this investigation, to contain no aliphatic sulfides. The absorbance of the iodine complex of this sample was determined at several sample concentrations and the apparent sulfide content calculated to be 0.016 weight %. This should represent a reasonable "interference value" for aromatics for most samples. If anything, it may be slightly high, as there is no reason to believe that no aliphatic sulfide sulfur was present.

On the basis of these studies, it is suggested that the interference due to aromatics may reasonably be assumed to be 0.01 to 0.02 weight % aliphatic sulfide sulfur. Referring to Table IV and Figure 9, it is apparent that this would constitute negligible interference for most crude oils and chromatographic fractions. However, until the aromatic content of a sample and its interference can be more precisely determined, it is not recommended that this method be applied to samples containing less than 0.05 weight % aliphatic sulfur.

Table IV. Sulfide Sulfur Content of Several Crude Oils

Crude Oil	Total Sulfur, %	Aliphatic Sulfide Sulfur, %	Relative Percentage of Sulfide Sulfur
Light Mara, Venezuela	1.91	0.45	24
Heavy Mara, Venezuela	4.67	0.79	17
Kuwait	2.46	0.61	23
Light Eastern, Venezuela	0.87	0.27	31
Heavy Eastern, Venezuela	2.40	0.78	31
Permian McElroy, West Texas	2.37	0.41	17
Coniferous sulfur, Kentucky	1.94	0.26	13
Santa Maria Valley, California	2.34	0.93	40
Wilmington, Calif.	1.39	0.82	59
North Ward Estes, Tex.	1.40	0.46	33

Interference Due to Sample Absorption. If a sample solution containing high concentrations of certain aromatic hydrocarbons is to be analyzed, interference attributed to background absorption may be encountered. Fortunately, this interference is greatest below 300 mμ. Above the analytical wave length, 310 mμ, interference owing to background absorption is not serious for most samples. As can be seen from Figure 5, stray light effects produce anomolous absorption below 300 mμ when the sample concentration is too high. By decreasing the sample concentration stray light effects are reduced and a more typical

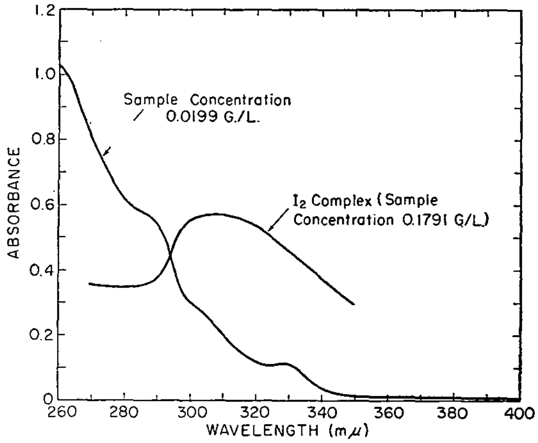

Figure 5. Ultraviolet spectra of chromatographic fraction 9 and its iodine blend

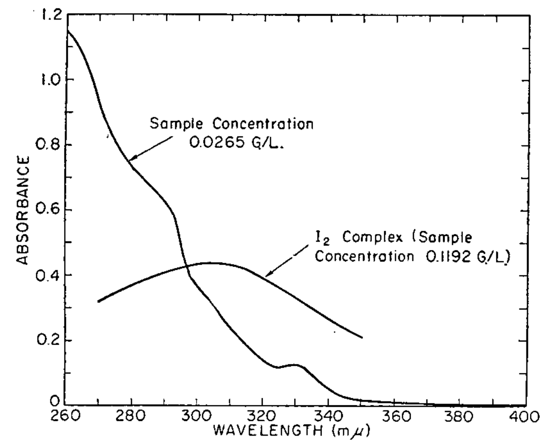

Figure 6. Ultraviolet spectra of chromatographic fraction 8 and its iodine blend

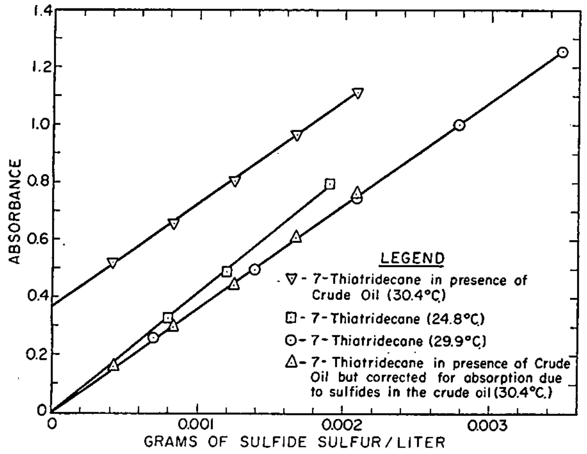

Figure 7. Absorptivity of 7-thiatridecane at an iodine concentration of 1 gram per liter under various conditions

spectrum is obtained, as illustrated in Figure 6. Even though background absorption for most samples is more intense than is usually encountered in routine spectrophotometry, reliable analytical results may be obtained with the use of the photomultiplier attachment.

Effect of Temperature. The Beckman DU spectrophotometer used for this investigation was not equipped with thermospacers for precise temperature control, but all measurements were made immediately after the solutions had been placed in the cell compartment, before any appreciable temperature change occurred. Tap water was circulated through the lamp housing to keep the cell compartment as near room temperature as possible. Therefore, any relationships concerning temperature reported here are based upon room temperatures at the time of measurement. From the data in Figures 7 and 8 and other data from the analysis of certain crude oils, it is estimated that the absorptivities decrease about 2 or 3% per degree Centigrade increase in temperature. This large coefficient is probably attributable to the temperature dependence of the equilibrium constant and very slightly to the thermal expansion of carbon tetrachloride. There is a significant difference between the percentage of sulfide sulfur for the Wilmington crude oil analyzed at 29.8° C. (Table V) and at 26° C. (Table IV) when the same absorptivity is used. A convenient reliable procedure for temperature correction when the spectrophotometer is not equipped with thermospacers consists of using the absorptivity of the 7-thiatridecane–iodine complex which is near 400 liters per gram-cm. at 26° C., at the same temperature at which the sample is analyzed. Figure 8 shows the variation of the absorptivity of the 7-thiatridecane–iodine complex with temperature.

Reproducibility of Method. A crude oil and a combination of several chromatographic fractions were analyzed for aliphatic sulfides at several different concentrations. The results are set forth in Table V. Reference to these results shows that the reproducibility is about 1 or 2%. The Wilmington crude was analyzed at 29.8° C. and the combined fractions at 25.5° C.

Recovery of Added Sulfide. Different amounts of 7-thiatridecane were added to a crude oil (Wilmington), and the procedure for the determination of aliphatic sulfides was applied to the mixtures. After correcting for the aliphatic sulfides present

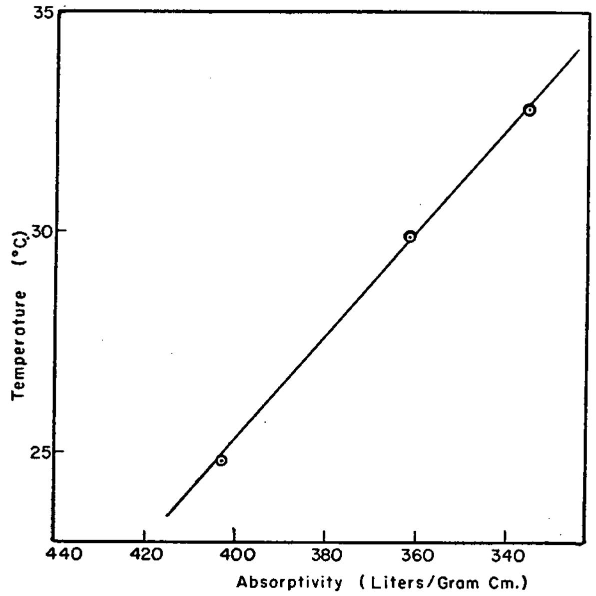

Figure 8. Effect of temperature upon complex formation between 7-thiatridecane and iodine

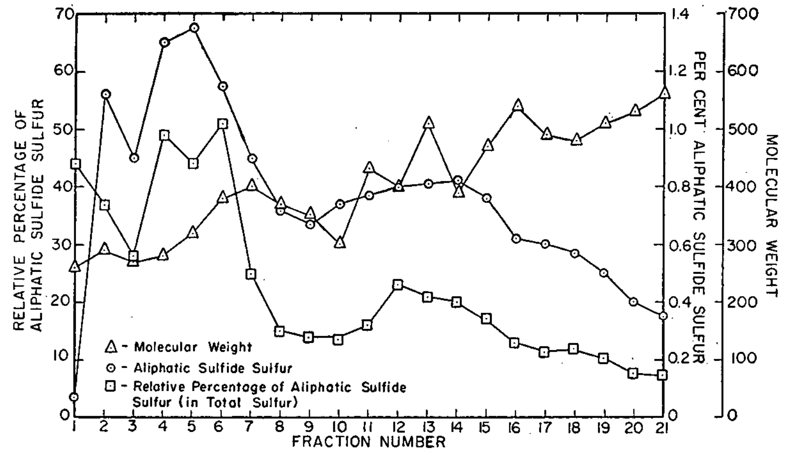

Figure 9. Aliphatic sulfide sulfur content and molecular weight of a series of chromatographic fractions from North Ward Estes (West Texas) crude oil

Table V. Reproducibility of Method Over Range of Sample Concentrations

Concentration of Sample in Iodine Blend, Grams/Liter	Sulfide Sulfur Found, %
Mixture of Several Chromatographic Fractions[a]	
0.0371	0.674
0.1112	0.683
0.1483	0.705
0.1854	0.698
	Average 0.69 ± 0.01
Crude Oil, Wilmington Field, California	
0.0678	0.775
0.1356	0.753
0.2035	0.748
0.2713	0.740
0.3391	0.750
	Average 0.75 ± 0.01

[a] Aliphatic sulfide sulfur content, calculated from experimentally determined aliphatic sulfide sulfur content of each fraction used in preparing the mixture, was also 0.69%.

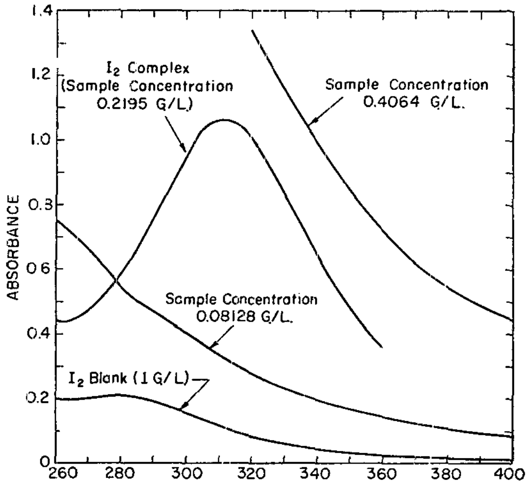

Figure 10. Spectra of Santa Maria Valley, Calif., crude oil and its iodine blend

in the crude oil, complete recovery of the added sulfides was obtained (Figure 7). The average absorptivity for 7-thiatridecane alone (at 29.9° C.) was 362 ± 4 liters per gram-cm. while the absorptivity for 7-thiatridecane in the presence of crude oil (at 30.4° C.) was 363 ± 9 liters per gram-cm.

Effect of Time in the Dark. The rapid decrease in the absorbance of the iodine blend when placed in the dark (cell compartment), as reported by Hastings, was not observed under the conditions used in this investigation. Table VI gives some absorbance *vs.* time in the dark data for several aliphatic sulfide-iodine blends. Variations in absorbance are of the order of magnitude of the accuracy of instrumental measurement.

Estimated Over-all Accuracy of Method. Considering the estimated interferences from formation of aromatic hydrocarbon-iodine complexes and from sample absorption and the uncertainty in establishing an average absorptivity for the iodine complexes of aliphatic sulfides likely to be present in crude oil, the accuracy of the method is believed to be of the order of 10%. This degree of uncertainty must be considered tolerable in the analysis of extremely complex samples such as crude oil.

RESULTS

Results of the analysis of a series of chromatographic fractions from North Ward Estes (West Texas) crude oil for aliphatic sulfides are shown graphically in Figure 9. These fractions were obtained by Clarence Karr, Jr. (Multiple Fellowship on Petroleum of the Gulf Research & Development Co.), by means of a chromatographic technique which has not, as yet, been published. Typical ultraviolet absorption spectra of a sample and an iodine blend are presented in Figure 6.

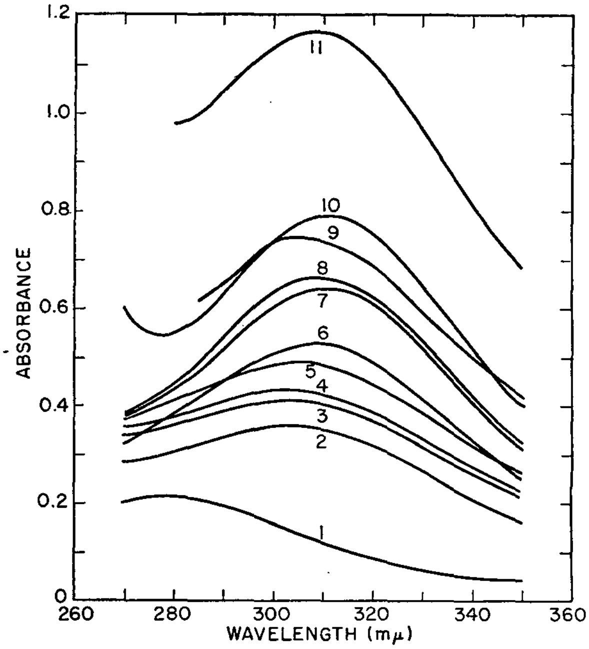

Figure 11. Typical spectra of crude oil–iodine blends

1. Iodine blank (1 gram per liter)
2. Light Mara, Venezuela
3. Light Eastern, Venezuela
4. Corniferous sulfur, Kentucky
5. Heavy Eastern, Venezuela
6. West Texas, Permian McElroy
7. Kuwait
8. Heavy Mara, Venezuela
9. North Ward Estes, West Texas
10. Light Mara, Venezuela
11. Wilmington Field, California

Typical spectra for a crude oil and a crude oil–iodine blend are illustrated in Figure 10. The spectra of the iodine blends of some additional crude oils are shown in Figure 11. The calculated aliphatic sulfide sulfur contents of these crudes are also given in Table IV.

The data in Table IV, although not extensive, indicate that the relative proportion of aliphatic sulfide sulfur is probably very similar for different crude oils from the same general field, regardless of the total sulfur content, as shown by a comparison of the

Table VI. Absorbance *vs.* Time in Dark Data for Several Aliphatic Sulfide-Iodine Complexes

Time in Dark, Min.	Absorbance at 310 mμ					
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]
0	1.240	0.610	0.930	0.528	0.613	0.672
2	1.239	0.610	0.930	0.528	0.612	0.670
4	1.239	0.612	0.930	0.529	0.612	0.670
6	1.239	0.612	0.930	0.529	0.612	0.667
8	1.239	0.612	0.935	0.530	0.615	0.668
10	1.238	0.612	0.935	0.530	0.617	0.668
15	1.240	0.612	0.935	0.532	0.617	0.668
20	1.237	0.611	0.930	0.532	0.617	0.667

[a] Thiacyclopentane, API 48A.
[b] 2-Methylthiacyclopentane, API 48A.
[c] 2,2,4,4-Tetramethyl-3-thiapentane, API 48A.
[d] 2,8-Dimethyl-5-thianonane, Eastman Kodak Co.
[e] 7-Thiatridecane, Eastman Kodak Co.
[d] 7-Thiatridecane, Eastman Kodak Co.
[f] 8-Thiapentadecane, Eastman Kodak Co.

relative percentages of sulfide sulfur in the Light Mara and Heavy Mara of Venezuela, the Light Eastern and Heavy Eastern of Venezuela, and the Santa Maria Valley and Wilmington crudes of California.

This method should prove valuable in following the course of the isolation or concentration of aliphatic sulfides from crude petroleum by techniques such as chromatography and thermal diffusion, and should be helpful in characterizing crude petroleum prior to refinery operations.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Multiple Fellowship on Petroleum sustained at Mellon Institute by the Gulf Research & Development Co., which has supported the work described herein, for permission to publish this material.

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RECEIVED for review August 12, 1954. Accepted December 15, 1954.

Manual and Continuous Recording Attachments for the Beckman Model DU Spectrophotometer

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The Beckman Model DU spectrophotometer has found wide application in the field of visual and ultraviolet spectrophotometry. To reduce the time required to plot a spectral curve with this instrument, the authors built a manual plotting attachment which would record the data from the spectrophotometer directly on a plotting paper. This saves time but suffers from the inherent disadvantages of point-by-point plotting in that one can only obtain spaced points on the curve. The continuous recording attachment described in this paper has the advantage of giving a complete record of the per cent transmittancy at all wave lengths through which the instrument is operated.

THE Beckman Model DU spectrophotometer was first described by Cary and Beckman in 1941 (*5*). Since that time about 10,000 instruments have been built for use in the field of visual and ultraviolet spectrophotometry. The authors believe this large use has resulted because, as stated by Gibson and Balcom (*9*) of the U. S. Bureau of Standards in 1947, "The instrument is capable of precise and accurate measurement of spectral transmittance or transmittancy."

The Beckman Model DU instrument was one of the instruments used by Haupt (*10*) of the Bureau of Standards for establishing an alkaline solution of potassium chromate as a transmittancy standard in the ultraviolet. This solution along with a Tropex neutral glass filter was used by Brode, Gould, Whitney, and Wyman (*2*), also of the Bureau of Standards, to carry out a comparative survey of Beckman Model DU instruments located in a number of cooperating laboratories. They report that, in general, the results show good agreement among the spectrophotometers. A paper by Caster (*6*) presents and discusses data on the variability in the Beckman Model DU spectrophotometer and refers to a number of publications which report the precision of both the instrument and the over-all analytical methods in which the instrument is used. The performance of the Beckman Model DU instrument, as indicated by these reports and the experience in many laboratories, has made it a required analytical tool for chemical research and process control. Its reasonable cost has also made it available within the budget of the average laboratory.

Because of the wide availability of the Beckman Model DU, a number of systems for the automatic recording of spectral data which incorporate this instrument have been devised and described in the literature. Coor and Smith (*7*) have described an automatic recording spectrophotometer which gave high-speed recording of per cent transmittancy. This instrument was reported to have worked satisfactorily for a number of years, but it has the disadvantage that the "correction device" is not readily adjustable. The Beckman Model DK recording spectrophotometer is based on the design originally made by Kaye, Canon, and Devaney (*11*) and Kaye and Devaney (*12*) of the Tennessee Eastman Co. With change in light sources and detectors and by a change in the Beckman Model DU Spectrophotometer itself, it is possible to cover the range of 200 to 2700 mμ. Etzel (*8*) has also decribed a single detector split-beam automatic recording instrument which incorporated the Beckman Model DU spectrophotometer. Per cent transmittancy versus wave-length data have been recorded automatically in the region 205 to 400 mμ with the hydrogen lamp as a source and 320 to 700 mμ with a tungsten lamp as a source.

Beckman (*1*) has described a means of recording for process control the per cent transmittancy at a set wave length using the Beckman Model DU spectrophotometer as a source of monochromatic light. Cahn and Gale have described (*4*) the Beckman automatic operator, which can be attached to the Beckman Model DU spectrophotometer to produce point-by-point plots. The time for obtaining a normal curve of 22 points is about 3 minutes, and for a curve of 400 points about 60 minutes. The Model DU spectrophotometer can also be used for manual operation by the usual procedure. Recently, Müller (*14*) described the Process and Instruments recording spectrophotometer which also incorporates the Beckman Model DU monochromator. A tenfold increase in resolution and low scattered light over the manual operation is claimed through the use of a photomultiplier. Curves can be drawn at speeds of 5 to 50 minutes for the spectrum of 215 to 700 mμ. The apparatus referred to above indicates the importance of tools of this type. In this article two attachments are described, which have been built and found useful in the authors' laboratories.

MANUAL PLOTTING ATTACHMENT

The relatively time-consuming nature of taking and recording data from the Beckman Model DU led the authors to build a manual plotting attachment (*3*) which would record the data from the spectrophotometer directly on plotting paper as soon as the balance was obtained. Figure 1 shows a photograph of the attachment as combined with the Beckman Model DU. Figure 2 shows the same instrument with the cover removed.

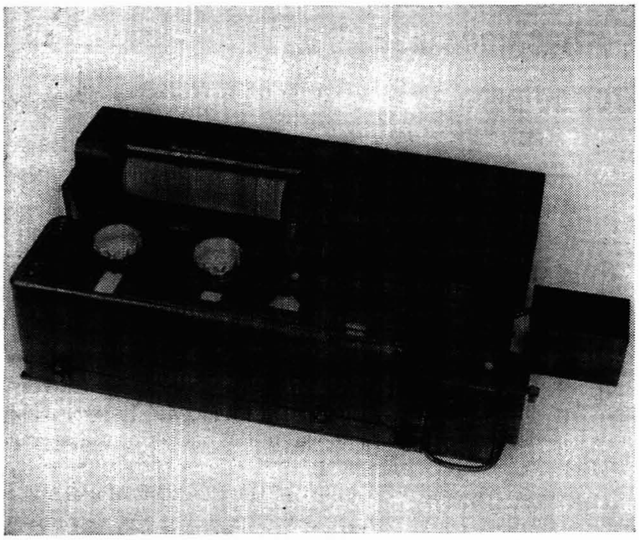

Figure 1. Manual plotting attachment combined with Beckman Model DU spectrophotometer

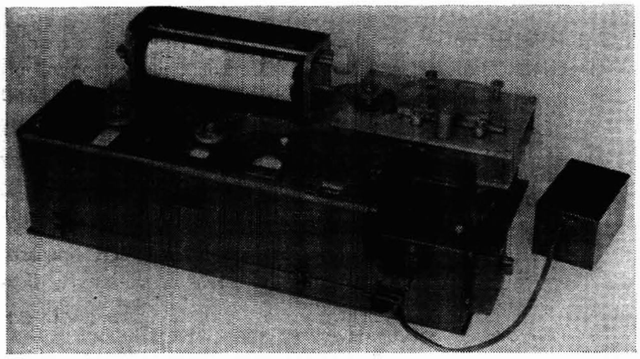

Figure 2. Manual plotting attachment with cover removed

In Figure 1 the large fluted aluminum knob on the left controls the change in wave length and is coupled by cable with the rotating drum. The wave-length cam within the spectrophotometer itself has been replaced with another cam, so that the wave-length abscissa on the plotting paper will be in octaval, or log log wave length. This octaval wave-length scale has been used on a number of laboratory spectrophotometers and has been found useful when ultraviolet, visual, and infrared spectra overlap or are joined together. It applies the usual advantage of log plotting to the wave-length ordinate. The direct coupling locates the pen at the same wave-length point at which the wave-length dial of the spectrophotometer is set. Hence, it is not necessary to adjust the dial of the spectrophotometer precisely to a given wave-length value before each reading, as is the usual case when the data are being recorded for later use. In actual practice, the wave-length points which are taken are dictated by the complexity of the curve being measured, the choice of wave-length spacing being made by the operator who moves the knob to obtain the wave-length position desired.

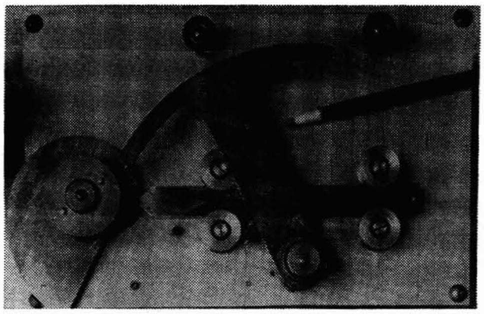

Figure 3. Detail of cam mechanism of manual plotting attachment

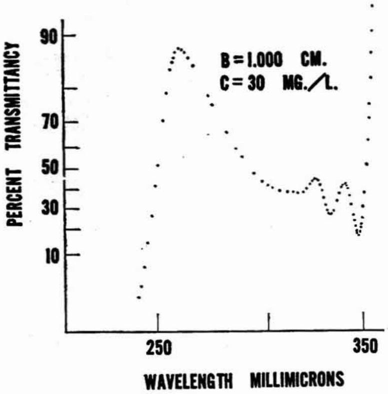

Figure 4. Ultraviolet spectrum of chromotropic acid in 0.1*N* hydrochloric acid plotted by manual plotting attachment

The large fluted aluminum knob on the right in Figure 1 is directly connected to the transmission dial of the spectrophotometer. By means of cables this is coupled to a cam mechanism shown in the upper right of Figure 2 and then to the horizontal motion of the pen mechanism. The cam mechanism shown in greater detail in Figure 3 changes the ordinate from one linear in transmittancy to one linear in log absorbancy. A description of the usefulness of the log absorbancy type of plot can be found in the book by Mellon (*13*).

In operating the manual plotting attachment, the instrument is balanced in the usual manner employed with the Beckman Model DU. After proper balance has been obtained, the push-button switch mounted on top of the cover just to the right of the transmittance knob is closed momentarily. This excites a

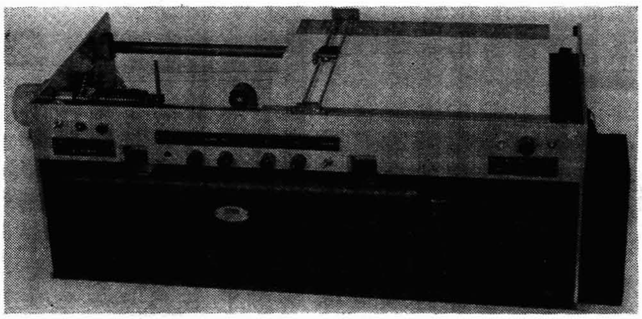

Figure 5. Over-all view of continuing recording instrument with Beckman DU monochromator in position

solenoid which causes the pen to strike the plotting paper, thus marking a dot on the paper at the proper wave-length and transmittancy values which correspond to the settings of the wave-length and transmittance dials of the instrument. The wave-length knob is moved to the next position, balance is carried out, and the next point is recorded. This is continued until enough points are obtained to produce a spectral curve over the range desired. This attachment does not shorten the time necessary to balance the spectrophotometer, but it eliminates the time for reading both wave-length and transmittance scales. In addition, it has the advantage that the spectral curve is obtained directly, plotted with the log absorbancy ordinate which allows for ready interpretation of spectral data. Thus considerable time is saved, and the data do not have to be subsequently plotted by hand from the recorded transmittancy and wave-length values.

Figure 4 shows the ultraviolet curve of chromotropic acid plotted by this manual attachment. The approximately 75 points were made in about 25 minutes or about 20 seconds per point. This point-by-point attachment was built inexpensively and is finding considerable use in the authors' laboratories, since it has speeded up the recording of entire spectral curves plotted with an ordinate which is desired for use in spectrophotometry.

CONTINUOUS RECORDING ATTACHMENT

Automatic continuous recording has many advantages over the point-by-point method of the Beckman Model DU or the manual point-by-point plotting attachment. It gives a complete record of the per cent transmittancy at all wave lengths through which the instrument is operated. In view of the advantages, one of the authors (C. W. Warren) proposed the general optical and electronic layouts of an automatic attachment and several physical modifications have been built in the American Cyanamid laboratories at Bound Brook. In this paper the authors' latest continuous recording attachment for the Beckman Model DU spectrophotometer is described from the point of view of construction, operation, and performance.

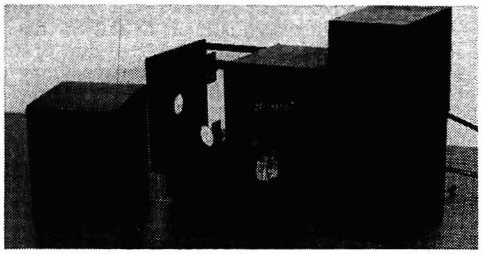

Figure 6. Unit containing photomultipliers, cell chambers, mirrors, and cylindrical shutter attached to end plate of monochromator

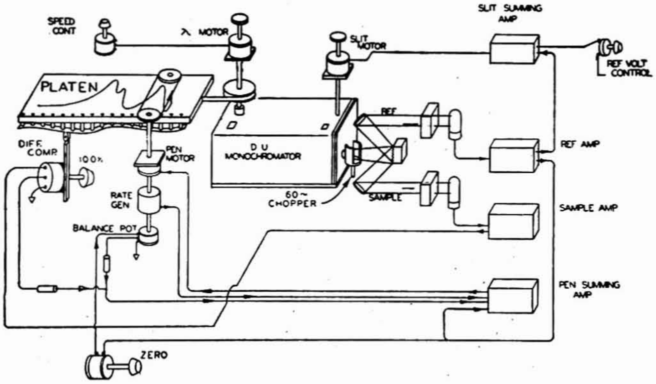

Figure 7. Schematic drawing of continuous recording attachment

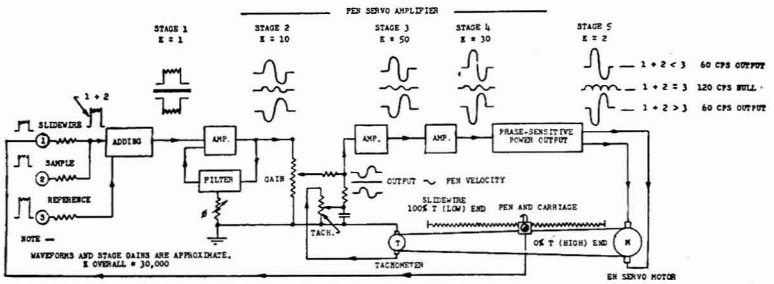

Figure 8. Block diagram of ratio-recording system

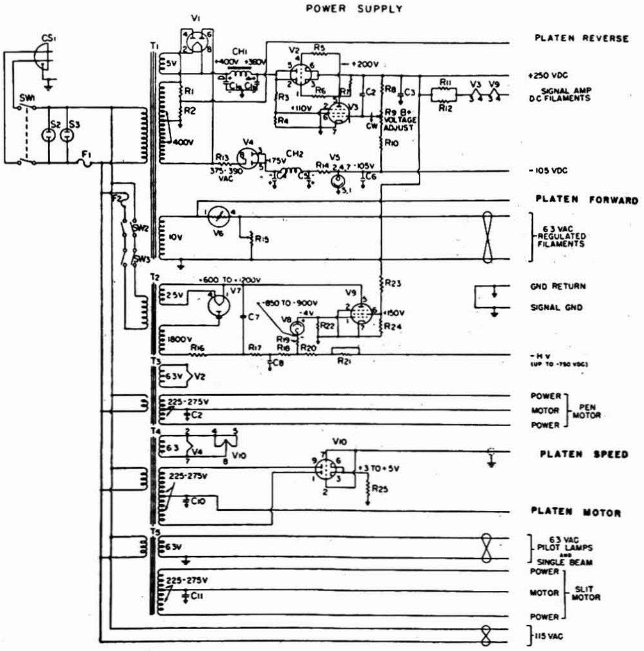

Figure 9. Power supply for ratio recording system

Part No.	Description	Part No.	Description
R_1	680 K, 1 watt	C_1	40-40 mfd., 450 volts (Mallory FP-238)
R_2	33 K, 1 watt	C_2	0.5 mfd., 600 volts (C-D PJ 6P5)
R_3	47 K, 2 watt	C_3	0.5 mfd., 600 volts (C-D PJ 6P5)
R_4	22 K, 2 watts	C_4	30 mfd., 350 volts (Mallory FP-135)
R_5	560 ohms	C_5	30 mfd., 350 volts (Mallory FP-135)
R_6	560 ohms	C_6	0.1 mfd., 400 volts (C-D PJ-4P1)
R_7	1 meg.	C_7	1.0 mfd., 1500 volts (C-D TJU-15010)
R_8	100 K	C_8	1.0 mfd., 1000 volts (C-D TJU-10010)
R_9	50 K (IRC 6104425 A 423) voltage adjust	C_9	1.0 mfd., 400 volts (C-D PJ-4W1)
R_{10}	47 K	C_{10}	1.0 mfd., 400 volts (C-D PJ-4W1)
R_{11}	2500 ohm, 20 watts (Ohmite Brown Devil)	C_{11}	1.0 mfd., 400 volts (C-D PJ-4W1)
R_{12}	2500 ohm, 20 watts (Ohmite Brown Devil)	V_1	5R4GY
R_{13}	2 K, 10 watts (Ohmite Brown Devil)	V_2	6AS7G
R_{14}	1 K, 10 watts (Ohmite Brown Devil)	V_3	12AU6
R_{15}	100 ohm, 10 watts (adjustable Ohmite)	V_4	6X5
R_{16}	56 K	V_5	OB2
R_{17}	100 K	V_6	Amperite
R_{18}	100 K	V_7	2X2
R_{19}	330 K	V_8	Victoreen 5841
R_{20}	150 K	V_9	12AU6
R_{21}	100 K	V_{10}	5687
R_{22}	120 K	T_1	Sterling T-1378A
R_{23}	47 K	T_2	Thordarson T22R40
R_{24}	68 K	T_3	Stancor PM-8418
R_{25}	500 ohms, 5 watts	T_4	Stancor PM-8418
F_1	Alden 440-5FH, 5 amp.	T_5	Stancor PM-8418
F_2	Alden 440-5FH, $^3/_8$ amp.	CH_1	Chicago RC-12150
SW_1	DPST, on-off	CH_2	Thordarson T20C53
SW_2	DPST, interlock	S_1	C-JS-324-CCT-K
SW_3	DPST, interlock	S_2	Amphenol 61-F AC receptacle
PL_1	Cornish cord set	S_3	Amphenol 61-F AC receptacle

All resistors 0.5-watt ± 10% unless otherwise specified.

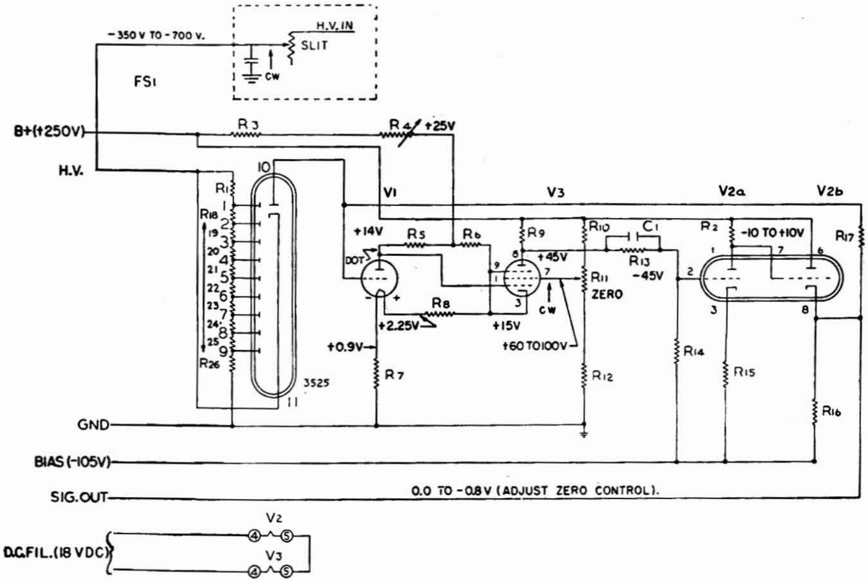

Figure 10. Signal amplifier for ratio recording system

Part No.	Description
R_1	68 K
R_2	120 K
R_3	20 K, 10 watts
R_4	5 K, 10 watts (Ohmite Dividohm)
R_5	100 K
R_6	1.2 K, 2 watts
R_7	82 ohm
R_8	1 K
R_9	270 K
R_{10}	27 K, 1 watt
R_{11}	50 K (Ohmite Type AB)
R_{12}	33 K
R_{13}	3 meg., 1% (IRC-DCF)
R_{14}	2 meg., 1% (IRC-DCF-1W)
R_{15}	27 K, 1 watt
R_{16}	10 K, 5 watts
R_{17}	100 meg., 5% (Victoreen)
R_{18}–R_{26}	100 K
C_1	0.005 mfd., 200 volts (Gudeman XFH-2535)
V_1	Electrometer tube (Victoreen 5803)
V_2	12AU7
V_3	5879
FS_1	Fanning strip (C-J 7-160-A-R)
	1P28 photomultiplier tubes (matched pair)

All resistors ±10%, 0.5-watt unless otherwise specified.

CONSTRUCTION AND OPERATION

Figure 5 shows the over-all view of the instrument with the Beckman monochromator in position.

The regular Beckman cell chamber and photocell compartment have been removed from the Model DU, but the Beckman hydrogen or tungsten source is retained. The separate monochromator portion without any electrical circuits of the Beckman Model DU was obtained for use with the recording attachment, but the attachment can be placed directly on the regular Model DU spectrophotometer, since the original electrical circuits are not used with the attachment. In placing the recording attachment on the Model DU, only two new mechanical connections are necessary. The wave-length drive is connected to the wave-length knob and the slit drive to the slit knob on the Beckman Model DU. The cell chamber and photocell compartment of the original Model DU are removed and instead there is attached to the monochromator end-plate a unit containing the photomultipliers, cell chambers, mirrors, and cylindrical shutter. This is shown with the cover removed in Figure 6. Figure 7 is a schematic view of the instrument.

Sample Cell and Detector Compartments. The beam from the exit slit of the monochromator is interrupted at 60 cycles per second in order to provide an alternating current signal for the electronic system. This beam is then split into sample and reference beams by means of a classical reflectance type of beam splitter which samples all portions of the beam for each of the two beams into which it is split. It is folded into two parallel beams by two concave spherical mirrors. These two beams pass through the reference and sample compartments which hold standard cylindrical-type absorption cells up to a maximum light-path length of 10 cm. The holder could be readily adapted to the use of the square 1-cm. cells if desirable. The beams then fall upon selected IP28 photomultiplier tubes which are mounted integrally with their associated voltage dividers and amplifiers. The Model DU spectrophotometer can be removed readily and be reassembled with the regular Beckman accessories for other uses since no changes were made to the spectrophotometer.

Ratio Recording. Ratio recording is accomplished by a unique circuit for which patent claims have been allowed (*15*). Figure 8 shows a block diagram of the recording system and Figures 9, 10, and 11 are the diagrams and parts lists of the power supply, signal amplifier, and pen or slit servo-amplifier, respectively. In the system under discussion a portion of the reference signal is added to the sample channel as necessary to raise the sample channel level to equal the reference channel. At zero per cent transmission all of the reference signal is used to oppose itself to raise the sample channel to the reference channel level and the noise in the reference channel cancels because of the opposite sign. The signal from the reference amplifier is fed to the summing amplifier and also across a balance potentiometer which is connected to the recording pen and pen drive motor. The differential compensator attenuates the sample signal and consists of a single potentiometer with two mechanical inputs arranged in differential fashion. One input is from an adjustable cam which is traversed by the wave-length drive. This input controls the flatness of the 100% line by compensating for spectral differences in detectors and mismatch of cells. The other input is a manual control which applies a bias to the angular position of the potentiometer body to raise or lower the over-all level of the 100% line. This input is called the balance control. A portion of the reference signal is then added to the sample signal by the balance potentiometer, and the sum is fed to the summing amplifier. If, at this point, the sum of the sample signal plus part of the reference signal does not equal the reference signal, the pen motor drives the pen and balance potentiometer as required for balance. A rate generator coupled to the pen balance motor develops a feedback signal which is used to stabilize the ratio recording pen system. A variable control in this feedback loop allows adjustment to suit the operator.

Slit Control. Continuous control of the slits of the monochromator to maintain signals of a reasonably constant level from the photomultipliers through the wave-length region being scanned is accomplished automatically by the slit servo. The reference signal is compared at the slit summing amplifier with a constant reference voltage, the slits being opened or closed by the slit drive motor as necessary to maintain the ratio between photomultiplier signal and reference voltage reasonably constant. Varying the reference voltage potentiometer varies the slit width and provides control of resolution. An over-all control of sensitivity is obtained by varying the high voltage supply to the photomultipliers.

Recording Platen. The recording platen is mechanically connected to the wave-length knob of the Beckman monochromator through cable drives and is driven by a continuously variable-speed motor. While any wave-length range can be chosen, it was decided to standardize on two, one covering 220 to 385 mμ and the other 300 to 750 mμ.

Adjustment of the Zero and 100% *T* Lines. The level of the zero line is adjusted electrically by means of a potentiometer control. The adjustment is made with radiation falling on the reference photomultiplier only, the shutter in the sample beam being closed. The zero line has been found to be stable at the level at which it is set.

The level of the 100% *T* line is also adjusted electrically by the balance control potentiometer with radiation falling on both sample and reference photomultiplier tubes. The position of the 100% *T* balance point was found to be stable at the level to which it is set. The 100% *T* line can be made straight over the entire spectral range by means of the adjustable cam differential compensator. The differential compensator cam is connected to the wave-length scanning mechanism. The adjustable cam consists of a flexible metal band which is backed up by screws which can be adjusted to determine its shape. These screws, which can be seen along the back of the platen in Figure 5 and along the front of the platen in Figure 7, are adjusted at each of these wave-length settings to give a correct 100% reading

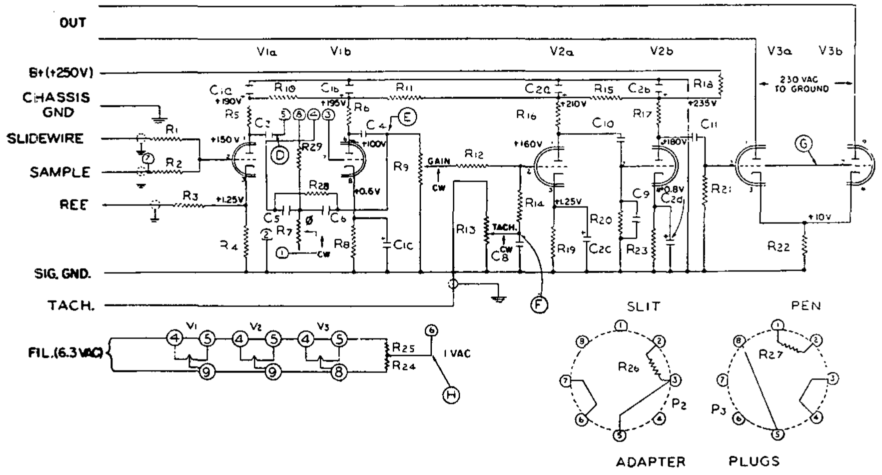

Figure 11. Pen or slit servo-amplifier for ratio recording system

Part No.	Description
R_1	1 Meg., 1% (IRC-DCF)
R_2	1 Meg., 1% (IRC-DCF)
R_3	68 K, 1% (IRC-DCF)
R_4	47 K, 1% (IRC-DCF)
R_5	680 K
R_6	180 K
R_7	10 K (IRC Type Q) (phase)
R_8	1 K
R_9	500 K (IRC Type Q) (gain)
R_{10}	47 K, 1 watt
R_{11}	27 K, 1 watt
R_{12}	390 K
R_{13}	10 K (IRC Type Q) (Tach)
R_{14}	1 Meg.
R_{15}	22 K, 1 watt
R_{16}	100 K
R_{17}	100 K
R_{18}	10 K, 1 watt
R_{19}	2.7 K
R_{20}	680 K
R_{21}	100 K
R_{22}	500 Ohm, 5 watts
R_{23}	1 K

All resistors ±10%, 0.5-watt unless otherwise specified.

Part No.	Description
R_{24}	220 Ohm
R_{25}	1 K
R_{26}	1 Meg.
R_{27}	22 K
R_{28}	2.7 Meg., 5%
R_{29}	150 K
C_1	*a.* 15 Mfd, 450 volts (Mallory FP-345.5) *b.* 15 Mfd., 450 volts (Mallory FP-345.5) *c.* 40 Mfd., 25 volts (Mallory FP-345.5)
C_2	*a.* 20 Mfd., 450 volts (Mallory FP-426) *b.* 15 Mfd., 450 volts (Mallory FP-426) *c.* 20 Mfd., 25 volts (Mallory FP-426) *d.* 20 Mfd., 25 volts (Mallory FP-426)
C_3	0.1 Mfd.
C_4	0.1 Mfd.
C_5	0.01 Mfd., 1%
C_6	0.01 Mfd., 1%
C_8	0.5 Mfd.
C_9	0.01 Mfd.
C_{10}	0.1 Mfd.
C_{11}	0.5 Mfd.
P_1	11-Pin plug (Amphenol 86RCP-11)
P_2	8-Pin plug (Amphenol 86CP-8)
P_3	8-Pin plug (Amphenol 86CP-8)
V_1	12AX7 Pen service, 12AU7 slit service
V_2	12AX7 Pen service, 12AU7 slit service
V_3	5687

under any desired cell conditions. As the instrument scans through the wave-length range, the follower on the adjustable cam actuates the differential compensator potentiometer, so that the balance point is maintained at 100% *T* over the entire spectral range being scanned. A 100% line which was flat to ±1.0% *T* can be plotted even with mismatched absorption cells in the sample and reference beams as shown in Figure 12. While this compensator accomplished the results shown in Figure 12, it was difficult to adjust and for routine laboratory use electrical compensation might be more desirable.

Scanning Time. The ultraviolet region from 220 to 385 mμ can be scanned in a continuously variable range of speeds. At the fastest speed, the scanning time is $1^2/_3$ minutes, while the slowest speed gives a complete ultra violet spectrum in approximately 1 minutes. Scanning speeds outside thi region can be obtained by changin the scanning gear ratios.

PERFORMANCE

Resolution. The resolution of th recording attachment was observe by measuring benzene vapor at th increasingly narrow slit widths whicl are possible as the photomultiplie dynode voltages are increased. Figures 13, 14, and 15 present thre curves of benzene vapor recorde with mechanical slit widths of 0.3 0.1, and 0.033 mm. at 250 mμ. Th slit widths at other wave lengths ar shown in Table I. The slit widths o 0.3 mm. used in Figure 13 are representative of the smallest slit use within the workable sensitivity rang of the normal Beckman photocel operation. Figures 14 and 15 show curves for increasingly narrower slit which are possible with the use o photomultiplier tubes. The secondar absorption peaks on the side of th main absorption bands definitely become resolved at the narrower slits Also, as the slits are made narrower the intensity of the narrow absorption bands is gradually increased The limit of the resolution of Beckman monochromator in the ultraviolet region appears to be easil approached without an excessive nois level.

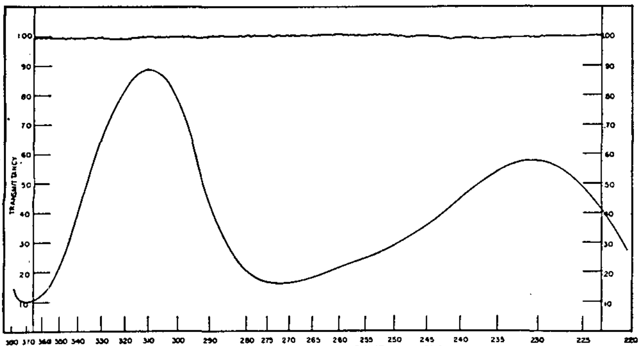

Figure 12. Ultraviolet absorption spectra of potassium chromate in 0.05*N* potassium hydroxide

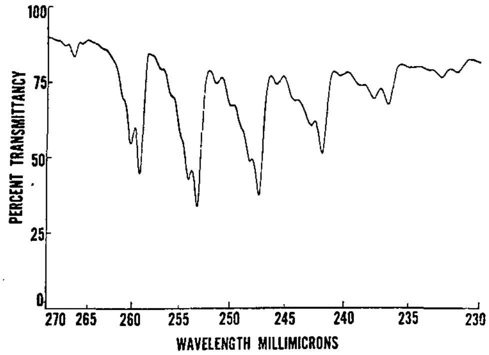

Figure 13. Spectrum of benzene vapor

Recorded with mechanical slit width of 0.3 mm. at 250 mμ

Table I. Slit Width Settings

Wave Length	For Curve on Figure 8	Figure 9	Figure 10
270	0.23	0.080	0.024
260	0.26	0.085	0.029
250	0.30	0.10	0.033
240	0.37	0.12	0.040
230	0.48	0.17	0.053

Linearity of Photometric Scale. A series of National Bureau of Standards calibrated gray glass filters was measured at a wave length of 530 mμ. Table II presents the comparison of the measured values with the calibrated values for each of the twelve filters or combinations. These data confirm the linearity of the photometric scale.

Table II. Photometric Scale Checked with Neutral Gray Filters

Calibrated Value	Value Found	Difference
2.0	2.0	0.0
3.8	3.7	−0.1
7.1	7.1	0.0
10.0	10.0	0.0
19.1	19.1	0.0
26.5	26.9	0.4
30.3	30.3	0.0
37.0	37.6	0.6
41.2	41.5	0.3
52.2	52.7	0.5
55.8	55.8	0.0
71.4	71.3	−0.1

Stability during Continuous Operation. The balance point at 100% T was found to remain fixed within ±0.5% during 18 hours of continuous recording at a fixed wave length of 300 mμ with air in the sample and reference beams as seen in Table III. As was shown in another test the balance point is little if any affected by line voltage changes as great as 30 volts or by small frequency changes. It is believed that the relatively small variations which occurred during the continuous operation test are more likely to be due to changes in ambient temperatures.

Reproducibility. The reproducibility of plotting a spectrum was checked by the use of the ultraviolet curve of benzene in iso-octane solution. The ultraviolet curve was plotted on the same plotting paper repeatedly, first at slow and then at fast scanning speed. The slow speed was at the rate of 14.5 minutes for the ultraviolet range of 385 to 220 mμ, while the fast speed was 2 minutes. The curves were found to be superimposed over each other within the width of the pen line, or approximately 0.1% T as shown in Figure 16, which shows that the instrument can be operated satisfactorily at high speeds.

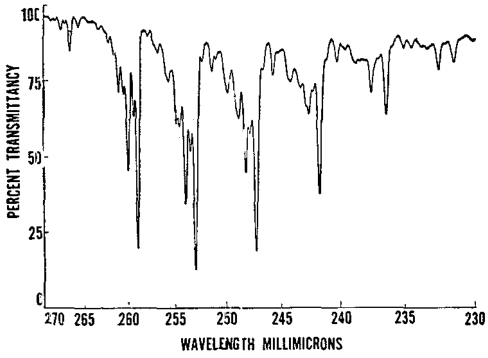

Figure 14. Spectrum of benzene vapor

Recorded with mechanical slit width of 0.1 mm. at 250 mμ

Table III. Continuous Stability Run

Hours	% Transmittance
0	100.0
2	99.8
4	100.1
6	100.4
8	100.5
10	100.3
12	100.4
14	100.2
16	100.2
18	100.0
Maximum variation	±0.5%

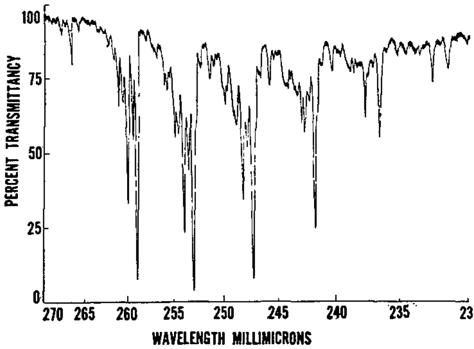

Figure 15. Spectrum of benzene vapor

Recorded with mechanical slit width of 0.033 mm. at 250 mμ

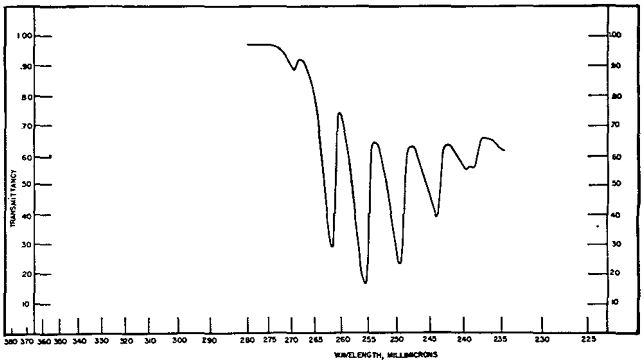

Figure 16. Ultraviolet absorption spectra of benzene in iso-octane showing superposition of curves run at fast and slow scanning rates

Fast rate, 2 minutes
Slow rate, 14.5 minutes for ultraviolet range 385 to 220 mμ

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RECEIVED for review February 16, 1954. Accepted January 3, 1955. Presented before the Division of Analytical Chemistry at the 124th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill.

Thiocyanate Spectrophotometric Analysis of Titanium, Vanadium, and Niobium

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The formation of the thiocyanate complexes of the elements of subgroups IV and V has been critically examined. There was little or no difficulty in adjusting these elements to the correct valence state. However, it was found relatively difficult to establish that the elements in the IV and V valence states were quantitatively reactive in a reasonable time. Thus, depending on the solution history, a given element formed the thiocyanate complex at varying rates. Special pretreatment of samples was necessary to obtain uniform and correct color development. The effects of variables of the color developing media—such as acid, salt, temperature, miscible solvent, and thiocyanate content—are discussed.

THE advantages of the thiocyanate-acetone medium in spectrophotometric analysis have been discussed in previous studies (*2*, *3*). This system has a particular advantage when applied to such easily hydrolyzed and/or polymerized elements as those of subgroups IV and V. Here the employment of high acidity and high organic to aqueous ratios in the complexing medium is necessary to keep the ionic and reactive species in solution and to obtain the high spectrophotometric sensitivity required for trace analysis of these elements. In this group only vanadium develops any appreciable absorbance in an aqueous medium. The intense absorbances are not associated with any one valence state. Thus, in the thiocyanate-acetone medium, titanium(IV), vanadium(III), and niobium(V) form very intense absorption spectra in the visible region, whereas titanium(III), vanadium(V), vanadium(IV), zirconium(IV), hafnium(IV), and tantalum(V) show relatively little absorbance. Of the three elements found to produce an intense absorbance in the thiocyanate medium, only niobium (V) has been recently applied analytically (*4*, *8*).

REAGENTS

After recrystallization from absolute methanol, reagent grade ammonium thiocyanate was dried and stored in a dry atmosphere (*3*). Acetone solutions were prepared and used on the same day; they were never kept at temperatures greater than 20° C.

Niobium oxide, C.P. grade, was obtained from the Fansteel Metallurgical Corp., Chicago. A special niobium metal sample obtained from the metallurgy division of this laboratory was also used. Both samples were analyzed spectrographically and found to contain approximately 0.5% tantalum, and less than a total of 0.2% of 29 other metal components.

Tantalum oxide was also obtained from Fansteel and analyzed spectrographically. Less than 0.1% niobium was found and 28 other metal components which were analyzed totaled less than 0.4%.

Stock solutions were prepared from C.P. fused vanadium metal, C.P. titanium dioxide, pure zirconium metal (hafnium-free, and more than 99% pure by spectrographic analyses), and a special sample of hafnium oxide of greater than 98% purity by spectrographic analysis.

The niobium metal, niobium oxide, tantalum oxide, zirconium metal, titanium oxide, and hafnium oxide were each dissolved in concentrated hydrofluoric acid in a platinum casserole. Sulfuric acid was added after solution was complete. Excess hydrofluoric acid was removed by fuming. Water was added to the cooled sulfuric acid solutions, and the samples were heated to white sulfur trioxide fumes. This procedure was repeated four times. The final 0.5 to 1.0 ml. of sulfuric acid solution in the platinum casserole was diluted to 1 liter with hydrochloric acid, tartaric acid, oxalic acid, or sulfuric acid to make the stock solutions desired. Similar solutions were prepared by a potassium bisulfate fusion in platinum ware. The prepared stock solutions ranged from 0.01 to 0.001*M* in total metal concentration.

Vanadium metal was dissolved in hydrochloric acid. Vanadium(V) stock solutions in 6*M* hydrochloric acid were prepared by oxidation with permanganate. Vanadium(IV) stock solutions were prepared by reduction of vanadium(V) with sulfurous acid.

The blanks employed throughout were prepared according to the standard procedure with only the metal sample replaced with the approximate equivalent of acid. With the proper reagents, the blanks were identical with water at 383 mμ or above. Below 350 mμ, the blank absorbance was generally too large and unreliable.

APPARATUS

A Beckman Model DU quartz spectrophotometer and a Cary Model 11 recording spectrophotometer were both used. One-centimeter quartz cells were used throughout. The counting equipment used to assay niobium-95 directly in solution consisted of a conventional decade scaler with a sodium iodide–thallium activated well-type crystal, packaged by Harshaw Chemical Co. The well was 0.5 inch in diameter by 1.5 inches deep. The crystal wall and base thickness was 0.5 inch. The entire crystal was encased in a cylinder with 4-inch thick lead walls to minimize the background count.

TITANIUM

Standard Procedure. The final titanium solution is reduced to 2.0 ml. of concentrated sulfuric acid by fuming. If hydrofluoric acid had been employed previously in the solution of the sample, repeated additions of 1.0 ml. of water and evaporation to sulfuric acid fumes will reduce the fluoride concentration below the interference level.

Transfer the final 2.0 ml. of concentrated sulfuric acid containing the titanium(IV) to a 25-ml. volumetric flask. Add 3.0 ml of water (used in making the transfer) and cool; add 2.0 ml. of concentrated hydrochloric acid, and then 15 ml. of 3.0*M* ammonium thiocyanate in acetone. Dilute to volume with water.

Shake the solution for 15 to 30 seconds and allow the acetone and aqueous phases to separate. Remove the aqueous phase by means of a transfer pipet placed under the acetone phase. It is not necessary to remove the aqueous phase quantitatively, and as much as 0.5 ml. is usually left in the volumetric flask.

To the remaining acetone thiocyanate phase containing the yellow titanium(IV) thiocyanate complex, add an additional 5.0 ml. of 3.0*M* ammonium thiocyanate in acetone and 1.0 ml. of 6*M* hydrochloric acid.

Dilute to 25 ml. with water, mix, and place in a 20° C. water bath for 5 minutes. The absorbance maximum is at 417 mμ. The concentration of titanium(IV) which gives an absorbance of unity at 417 mμ and 20° C. in 1-cm. cells is 1.28×10^{-5} mole per liter.

Discussion. The development of the titanium(IV) thiocyanate complex in acetone-aqueous medium is an exacting procedure, with the maximum color intensity being unobtainable without the use of mixtures of sulfuric and hydrochloric acids. Apparently both a strong complexing agent (sulfuric acid) for titanium(IV) and a very strong acid (hydrochloric acid) are needed, to keep the titanium(IV) in a complexed state which reacts readily with thiocyanate and to prevent the decomposition of the complex in the nonaqueous medium. Thus, with sulfuric acid alone, the development of the titanium(IV) thiocyanate complex in acetone-aqueous medium shows an initially high absorbance which steadily decreases with time. With hydrochloric acid alone, the absorbance is initially low, and usually shows a slow increase with time. These results are attributed to the fact that, in sulfuric acid medium, the titanium(IV) is complexed more strongly, possibly favoring a nonoxygenated $TiSO_4^{++}$ species. In hydrochloric acid apparently a larger percentage of the titanium is the titanyl species, or possibly more complex polymeric forms. This would account for the observed slow and partial color development with hydrochloric acid alone. The sulfuric acid, however, is not a strong enough acid to supply the necessary hydrogen ion in the acetone-aqueous medium to stabilize the titanium(IV) thiocyanate complex. The addition of hydrochloric acid immediately arrests the decrease in the absorbance of the titanium(IV) thiocyanate complex developed with sulfuric acid alone.

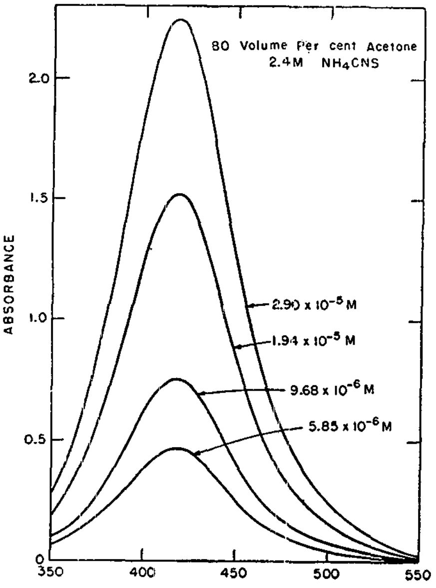

Figure 1. Titanium(IV) thiocyanate complex at 20° C.

Developed by standard procedure

Figure 1 shows the spectra of the titanium(IV) thiocyanate complex in the acetone-aqueous medium and the obedience of the total titanium(IV) concentration to Beer's law. The molar absorbance index is unusually high for the visible region, being 78,000 ± 800 at the 417 mμ peak at 20° C.

The color development of titanium(IV) thiocyanate complexes can be highly unreliable if the complex nature of titanium(IV) solutions is not recognized. The thiocyanate complexes are generally not regarded as strong complexes; hydration, hydrolysis, etc., will, under most conditions, interfere very effectively with the formation of the titanium(IV) thiocyanate complex, resulting in erratic color development. Titanium(IV) solutions are generally in a very complex state of partial hydrolysis or polymerization, which as yet is not very well understood. When small aliquots (25 to 100 μl.) of various $10^{-3}M$ titanium(IV) solutions are added to a given volume of the standard thiocyanate acetone medium kept at constant temperature, the solution will exhibit widely variable behavior in the initial absorbance, the rate of growth, and the final absorbance obtained. The reactive titanium present in the solutions examined was apparently indicated by the ratio of the absorbance obtained, and the known maximum calculated from the molar absorbance index for the thiocyanate medium employed.

To avoid effects of previous history, the titanium(IV) solutions have been fumed in concentrated sulfuric acid. Stock solutions of $0.01M$ titanium(IV) in $10M$ sulfuric acid were analyzed over a period of a year by the standard procedure and showed no appreciable decrease in the rate of formation of the correct absorbance. Eight molar hydrochloric acid solutions were considerably more erratic in behavior. The $10M$ sulfuric acid titanium(IV) stock solutions were used as a standard.

The rate of color development is an important factor for it decreases with a decrease in temperature (by a factor of 3 from 20° to 5° C.), with lower acidity, and with increased water content. With the standard procedure, the low water and high thiocyanate concentration during phase separation accelerates the rate of formation of the color, so that less than 5 minutes is required for the development of the maximum absorbance.

The rate of color development also varies markedly with the order of addition of the reagents. For example, when the titanium(IV) was added last as a 25 μl., $10M$ sulfuric acid solution to the 25 ml. of acetone-aqueous thiocyanate solution, adjusted to the same concentrations obtained by the standard procedure, the rate of color development appeared to be slower than that obtained by the standard procedure.

The intensity of the absorbance is affected markedly by the temperature. Figure 2 shows this effect on a typical solution. The volume contraction from 30° to 5° C. is only about 3%, whereas the observed increase in the absorbance over the same temperature range is about 30%. Repeated raising and lowering of the temperature on the solutions examined gave reversible absorbance changes.

VANADIUM

Standard Procedure. Prepare the sample for reduction by fuming in sulfuric, nitric, or hydrofluoric acids to remove organic matter, silica, and volatile matter. Continue fuming until not more than 0.5 ml. of concentrated sulfuric acid is present.

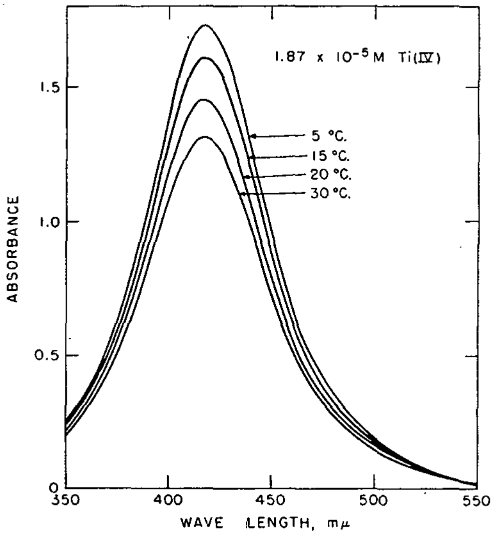

Figure 2. Effect of temperature on titanium(IV) thiocyanate complex

Developed by standard procedure

Add 1 ml. of concentrated hydrochloric acid, or if no sulfuric acid is present, add 2 ml.

Add approximately 200 mg. of stannous chloride, reagent grade, crystals and shake the solution until the crystals dissolve. Crystalline stannous chloride is added in order to avoid diluting the high acid concentration needed for rapid, complete reduction to vanadium(III). Allow the solution to stand at room temperature for 10 minutes.

Dilute the above solution with a few milliliters of distilled water. Add 15 ml. of $3.0M$ ammonium thiocyanate in acetone. Dilute to 25-ml. volume with water and mix thoroughly. The absorbance maximum is at 396 mμ. The color forms rapidly and may be read immediately.

Discussion. The vanadium(III) and vanadium(IV) thiocyanate spectra in aqueous solution have been studied recently by Furman and Garner (*5*). They reported that the vanadium(III) thiocyanate complex formed with 3.89 *F* ammonium thiocyanate has a molar absorbance index of 7200 at 400mμ. This species was applied analytically in the acetone-aqueous medium. However, the molar absorbance index in the acetone-aqueous medium at 1.80*M* ammonium thiocyanate is 11,750 at 396 mμ. No analytical applications of this complex have been found in the literature. Figure 3 shows the absorption spectra and the obedience of total vanadium(III) concentration to Beer's law. There is a small drop of 1.8% in the molar absorbance index at the highest concentration of vanadium ($1.52 \times 10^{-4}M$).

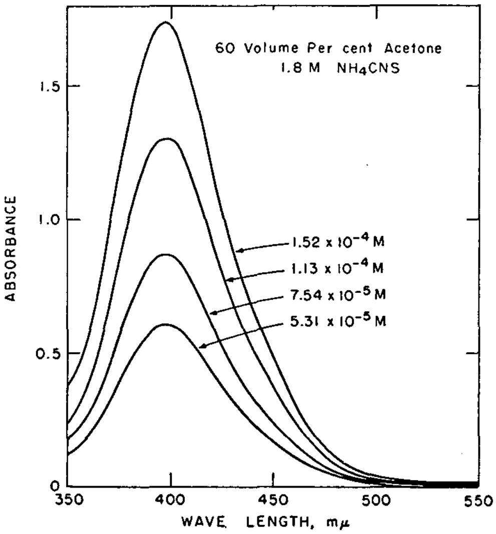

Figure 3. Vanadium(III) thiocyanate complexes

With the proper reduction procedure, the quantitative results of the vanadium analysis were excellent. Ten analyses on a sample of vanadium metal dissolved in 8*M* hydrochloric acid (to give a concentration of 0.0379*M*) and oxidized to vanadium(V) before applying the standard procedure gave a molar absorbance index of 11,750 ± 82. Ten analyses on a mixed-valent sample taken directly from a 6*M* hydrochloric acid solution after the metal dissolution gave identical results. Both solutions were analyzed frequently during a 6-month period, and no difficulty was encountered in obtaining quantitative results without prefuming or other pretreatment.

In approximately 6*M* hydrochloric acid the reduction to vanadium(III) with stannous chloride was complete in 2 hours, and in 10*M* hydrochloric acid, the reduction was complete in 10 minutes at room temperature. The stannous chloride concentration during reduction was approximately 3% by weight. Heating the solution appeared to make the results somewhat erratic probably because of reoxidation of vanadium(III) by oxygen in the hot solution. The vanadium(III), for all practical purposes, was stable in air in the 10*M* hydrochloric acid with excess stannous chloride present. Solutions containing a small excess of tin(II) and which were allowed to stand in the air for 15 hours developed the correct absorbance. Similar solutions shaken vigorously in air for 30 minutes also developed the correct absorbance.

Furman and Garner (*5*) have made an approximation of the first stability constant of vanadium(III) thiocyanate complex in solutions of ionic strength 2.5.

$$V^{+++} + CNS^{-} \longrightarrow VCNS^{++}, K \approx 100$$

Although the species in the acetone medium is undoubtedly a higher complex, possibly $V(CNS)_6^{---}$ or mixed complexes, the thiocyanate concentration must be kept high and constant to obtain maximum sensitivity and accuracy. Figure 4 demonstrates this point quantitatively and also shows the importance of the acetone. It is difficult to separate completely the effects of acetone in the color developing medium on hydration and on various hydrolysis reactions. However, in the case of uranium(VI), iron(III), cobalt(II), and vanadium(III), a large part of the effectiveness of the acetone is due to a decrease of competition of water of hydration with the thiocyanato ligand for coordination positions around the metal ion. The color stability was excellent. Vanadium solutions examined for more than 2 hours in ground-glass-stoppered cells showed no more than the usual instrument variations, 0.35%. In making stability studies, care must be taken to avoid loss of acetone by evaporation. Ground-glass-stoppered cells and water-cooled (20° C.) cell chambers were always employed. The effect of temperature on the absorbance of vanadium(III) thiocyanate was not so pronounced as titanium(IV) thiocyanate shown in Figure 2. Vanadium(III) thiocyanate complex showed about one fourth this variation.

NIOBIUM

Standard Procedure. EXTRACTION OF NIOBIUM FLUORIDE. The extraction procedure need be used only for the separation of niobium from other metals.

In a 100-ml. plastic, graduated cylinder, dissolve 8 grams of ammonium fluoride in 60 ml. of water. Add 10 ml. of concentrated hydrofluoric acid followed by 20 ml. of concentrated sulfuric acid, and dilute the solution with water to 100-ml. final volume. Then bring this solution to room temperature and equilibrate it with an equal volume of methyl ethyl ketone by shaking in a polyethylene bottle.

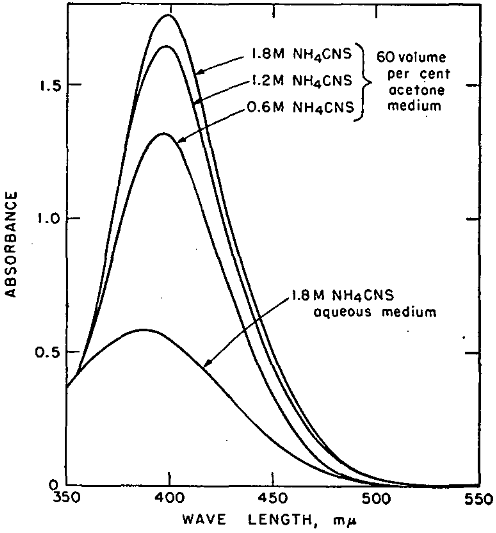

Figure 4. Effect of ammonium thiocyanate and acetone on vanadium(III) color formation

Add water, ammonium fluoride, concentrated hydrofluoric, and concentrated sulfuric acid to the niobium sample, in a minimum volume, so that their concentrations are the same as in the aqueous phase used to equilibrate the methyl ethyl ketone. It is usually convenient to restrict the volume extracted to 10 ml. Add an equal volume of equilibrated methyl ethyl ketone to the aqueous solution, and mix for 5 minutes to ensure equilibrium.

Transfer the organic phase (transfer pipet) to a platinum dish and make a second extraction from the aqueous phase with an equal volume of equilibrated methyl ethyl ketone. Add the second organic phase to the first in the platinum dish and evaporate at a moderate rate to prevent spattering and excessive charring.

When most of the methyl ethyl ketone has evaporated, add to the tarry mass 1 ml. of concentrated nitric acid, followed by 1 ml. of concentrated sulfuric acid. Increase the heat, and add carefully successive portions of concentrated nitric acid, as needed, to remove the color of the remaining organic matter. Finally, evaporate to dense white fumes of sulfur trioxide.

Reactive niobium will develop the correct absorbance immediately. But some nonreactive niobium solutions will develop the correct absorbance in a reasonable time (5 to 60 minutes). Examination of the color stability for approximately 10 minutes should be routine in a careful analysis. It is essential that all niobium solutions of uncertain history be freshly fumed in sulfuric and nitric acids before color-development. Addition of fluoride ion before fuming will also ensure the correct color development in solutions containing silicates and fluosilicates. The extraction procedure if used will provide this treatment. However, when the extraction procedure is not used, the color development should be preceded by the following treatment:

Place the niobium sample in a platinum dish, add 0.5 ml. of concentrated hydrochloric acid and 0.5 ml. of concentrated sulfuric acid, and heat the solution to dense white fumes. Also, add concentrated nitric acid to solutions containing organic matter as described in the extraction procedure before fuming in sulfuric acid.

Dilute the cooled sulfuric acid solution with 1 ml. of water just before transferring to the color-developing medium.

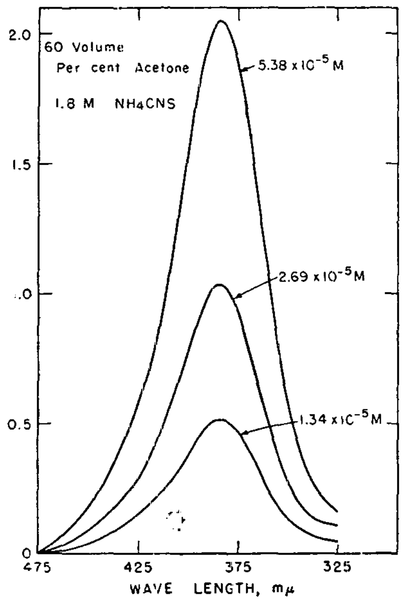

Figure 5. Niobium thiocyanate complex

Developed by standard procedure

Color Development of Niobium Solutions. Add 2 ml. of concentrated hydrochloric acid to a 25-ml. volumetric flask, followed by 15 drops of a clear 10% stannous chloride solution in 6*M* hydrochloric acid. Add 15 ml. of 3.0*M* ammonium thiocyanate in acetone. Add approximately 1 ml. of water and mix the solution. Transfer the cooled niobium sulfate solution from the platinum dish into the flask and dilute to volume with distilled water.

Shake the solution until it is well mixed and free of any precipitate. Read the absorbance at 383 mμ in glass-stoppered 1-cm. silica cells. Allow 15 minutes for maximum absorbance to develop at 20° C. Molar absorbance index was 38,000 ± 400.

Discussion. Quinquevalent niobium develops a stable and very sensitive, colored complex with thiocyanate in many organic or mixed organic-aqueous media. The complex may be extracted into a variety of organic liquids. Two recent papers by Freund and Levitt (*4*) and Lauw-Zecha, Lord, and Hume (*8*) review the important literature on the niobium thiocyanate analytical method.

There is no difficulty with valence adjustment, since niobium(V) is the only stable valence state of this element in aqueous solution. No evidence was found for abnormal stabilization of niobium(III) with any of the complexing ligands employed in this investigation, and the intense absorbance peak at 383 mμ may be assigned to a niobium(V) thiocyanate complex. Niobium(V) thiocyanate complex is relatively weak, and its absorbance is affected by changes in thiocyanate concentration, by hydrolysis or by hydration. The color-developing procedure was standardized and the maximum molar absorbance index was established for standard reactive niobium(V) solutions. Figure 5 shows the niobium thiocyanate spectra. The total niobium concentration follows Beer's law over a wide concentration range. In this procedure, the acetone to aqueous ratio is higher than in previous investigations on this element; this results in increased sensitivity, minimizes the effects of niobium hydrolysis, and reduces foreign anion interference to a considerably lower level.

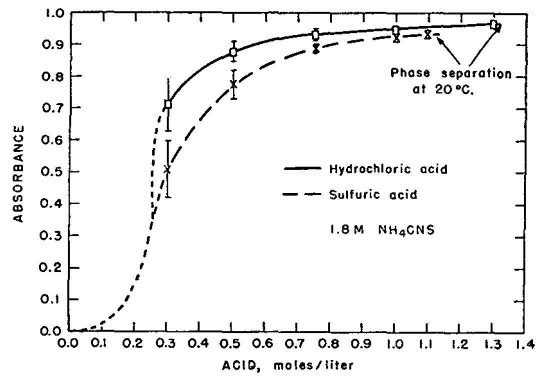

Figure 6. Effect of acid concentration on development of niobium thiocyanate in acetone-aqueous medium

Figure 5 shows the effect of sulfuric acid and hydrochloric acid concentrations on the color development. The decreased absorbance at low acid concentrations is associated with the partial hydrolysis of niobium(V) in the acetone-aqueous medium. The lesser effectiveness of sulfuric acid in this medium is associated with its weaker acidity rather than with its ability to complex niobium. At higher concentrations of sulfuric acid, the absorbance approaches very closely that achieved with hydrochloric acid. The hydrogen ion appears to play a more important role in the color development than does reduction of the water content in the medium, because it is possible to develop appreciable absorbance in aqueous thiocyanate solutions if the acidity is very high.

The effect of hydrolysis upon the niobium thiocyanate complex color development was demonstrated by using oxalic acid solutions of niobium and developing the niobium thiocyanate complex under varying conditions. A 0.1*M* oxalic acid solution of niobium(V) was prepared relatively salt-free by dissolving the wet washed freshly precipitated hydrated oxide in the dilute oxalic acid. A 0.5-ml. aliquot of this oxalate solution rapidly developed an absorbance of 1.00 when the standard procedure was applied. However, addition of a 0.5-ml. aliquot of this solution to 25 ml. of 3*M* ammonium thiocyanate–acetone solution with no added water or acid present produced a clear colorless solution. Addition of 6*M* hydrochloric acid became effective when a concentration of about 0.1*M* was reached (Figure 6), and the color developed as additional acid was added. The niobium remained in reactive forms in all of the above solutions, as indicated by the rapid color development in the standard medium when acid was added. This suggests that two distinct series of complexes exist in acid medium. The titanium(IV) (*10*) is also believed to have two similar series of complexes in acidic medium.

An acetone solution of the niobium thiocyanate complex was passed through a Dowex 2, 200-mesh, anion exchange column (previously equilibrated with acetone-thiocyanate), and a well-defined yellow band formed at the top of the column.

A summary of recent investigations of the two groups, titanium-zirconium-hafnium and vanadium-niobium-tantalum, shows that a graduation in the stability of the oxygen metal bond decreases with the heavier elements. When in solution, vanadium(V) and (IV) oxygenate, and they show little or no evidence for the formation of VX_n^{-n+4}. Zirconium (*1, 6*) hafnium, and tantalum (*7, 11*) show a preference for the ZrX_n^{-n+4} type complex, and the existence of the oxygenated species of these elements in solution is doubtful. Titanium(IV) (*10*) and niobium(V) (*6*) show evidence of both types of complexes in solution.

The effects of anionic interference on the color development are summarized in Table I. Some care must be exercised in interpreting the results of the interference tests, as the pH and aging of the solutions may play an important and unpredictable role in the reactions of the various anions with niobium(V). The compounds tested for interference were added to the aqueous niobium solution just prior to the color development.

Table I. Effect of Foreign Anions on Development of Niobium Thiocyanate Complexes

(Niobium concentration, 4.96 × $10^{-5}M$)

Interference	Molar Ratio, Foreign Ion/Nb	Error, %	Remarks
H_2SO_4	$\sim 10^5$	0.01	Sulfuric acid alone was unable to furnish a high enough hydrogen ion concn. 1.00 Ml. of concd. HCl also added
HBr	$\sim 10^5$	0.01	3 Ml. of 8M HBr used in place of same HCl concn. with no observable deviation
NH_4F	55 110	−1.6 −7.1	No detectable error at molar ratio of 30
H_2SiF_6	10	−50	Easily removed by adding excess HF and fuming in concd. H_2SO_4
NaOAc	1200	0.01	
K_2HPO_4	234	−3.0	Solution heated to point of fuming in H_2SO_4 before color development
Oxalic acid	180 400	−1.7 −21	No detectable error at molar ratio of 100
Citric acid	2600	−0.70	
Tartaric acid	2000	−0.50	Tartaric acid solutions showed slow color development.

It appeared that reactive forms of niobium(V) in aqueous solution were possible only with the aid of complexing agents. The criterion for reactivity of niobium(V) in solution was the ability of a niobium(V) aliquot to form the thiocyanate complex rapidly. To determine the reactivity of the niobium(V) in solution requires knowledge of the total concentration niobium(V) in the solution examined and the molar absorbance index of a 100% reactive niobium solution. The molar absorbance index was determined with standard solutions of niobium(V) as a complex fluoride, sulfate, oxalate, chloride, and tartrate. Each solution of niobium(V), when properly complexed, quantitatively produced the same molar absorbance index when the standard procedure was applied. The molar ratio of niobium(V) to complexing agent was such that there was no interference (Table I) to the described standard procedure. The complexed niobium(V) was added last to the adjusted thiocyanate medium and mixed, and the absorbance observed continually in the Cary recording spectrophotometer at 383 mμ within approximately 45 to 50 seconds of the initial mixing. When certain nonreactive forms of niobium(V) are employed, addition of the niobium(V) aliquot to strong acids prior to color development may increase the rate of formation of the niobium(V) thiocyanate complex and the final absorbance.

Fuming niobium with sulfuric acid and mixed sulfuric-hydrofluoric acids gave solutions which were very rapid and reproducible in color development. When potassium bisulfate melts of accurately weighed niobium metal or niobic oxide were dissolved in concentrated hydrochloric acid or 10M sulfuric acid, the solutions were also reproducible and rapid in color development. Potassium bisulfate melts of niobium which were dissolved in 0.1M oxalic acid to give a $10^{-3}M$ niobium(V) concentration rapidly developed 90% of the color and required approximately 10 minutes to obtain the stable and correct molar absorbance index.

Potassium bisulfate melts of either niobium metal or niobic oxide dissolved in 0.5M tartaric acid showed a more startling effect. There was essentially no color formed on mixing the niobium(V) with the thiocyanate, indicating the initial absence of the reactive niobium(V) species. However, the rate of color development was a first order reaction in niobium(V) with a specific velocity constant of 0.198 reciprocal minutes (Figure 7). This constant was obtained immediately after preparing the solution and remained the same for over a year of storage.

Although the reactions were always first order with respect to the niobium(V) concentration, the specific velocity constant varied with the tartaric acid concentration. Increasing the tartaric acid from 0.5 to 1.0M showed that approximately 50% of the niobium was initially in a reactive form in the higher tartaric acid concentration, probably as a $NbO(C_4H_4O_6)^+$ complex. The maximum molar absorbance index on tartaric acid solutions was the same as that obtained rapidly on sulfuric or hydrochloric acid solutions.

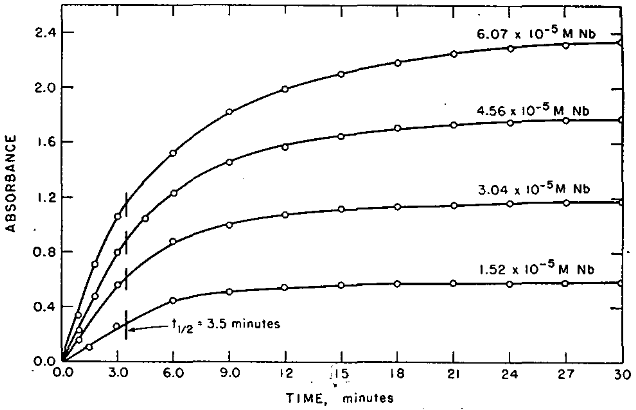

Figure 7. Rate of growth of niobium(V) thiocyanate complex

From 0.5M tartaric acid stabilized solution at 22° C.

Potassium bisulfate melts of niobium(V) dissolved in 0.2 to 1.0M citric acid showed no initial color development. These solutions differed from tartaric acid in that no formation of niobium(V) thiocyanate complexes was observed over a period of several hours. Colloids, as indicated by the Tyndall effect, were not evident in the tartaric or citric acid solutions.

The unreactive forms of niobium(V) formed in citric or tartaric acid were partially converted to reactive forms when allowed to stand in contact with 9M hydrochloric acid. This was also evident in the observation that the order of addition of the reagents made large differences in the rate of formation of the niobium(V) thiocyanate complexes. The lack or slowness of color development was associated with the failure of citrate and tartrate to complex the niobium completely, resulting in the formation of complex niobates similar to the vanadates. In tartaric acid, the first rapidly formed complex niobate species appeared to be stable, as indicated by the immediately reproducible first order specific velocity constant, the stability with time, and the zero initial reactive niobium concentration. The relative complexing ability indicated is niobium(V) oxalate >> tartrate > citrate.

It is concluded that niobium(V) complexes and NbO^{+++} or

$Nb(OH)^{5}{}_{\bar{x}}^{\;x}$ and its complexes are the reactive forms of niobium. Nonreactive forms are probably niobates and polymeric forms.

Solutions which were observed to contain niobium(V) colloid showed no color development when added to the thiocyanate-acetone standard medium. These solutions, and others in which the thiocyanate complex developed slowly or not at all, could be quantitatively converted to a reactive form by treating the sample in a platinum dish with concentrated hydrofluoric acid and fuming with sulfuric acid. Except when silicates were present, fuming with sulfuric acid or sulfuric-nitric acid mixtures was equally effective in converting niobium(V) quantitatively into reactive forms.

Table III. Extraction of Niobium from Metal Ions Which Interfere in Spectrophotometric Method

Interference	Niobium Concn., *M./L.*	Foreign Ion Concn., *M./L.*	Molar Ratio Foreign Ion Niobium	% Error[a] Without extraction	% Error[a] With extraction	Remarks
$NiCl_2$	2.30×10^{-5}	2.30×10^{-3}	100	44.0	0.00	
$FeCl_3$	2.30×10^{-5}	0.0167	725	500	0.00	% error estimated using $SnCl_2$, which cannot effectively eliminate all Fe^{+++} at 0.0167*M*
UO_2SO_4	2.48×10^{-5}	1.38×10^{-3}	55	2000	0.30	

[a] In each case, per cent of error was average of six experiments.

The development of the niobium complex does not appear to be particularly dependent on the chloride ion concentration, as previously suggested (*4*). The hydrogen ion activity undoubtedly acts to prevent hydrolysis of the metal thiocyanate complex. However, at higher concentrations both the hydrogen ion and added alkali metal salts appear to aid the development of the absorbance in proportion to their ability to orient or hydrate the water in the color developing medium. The salt and acid and added acetone or alcohol all contribute to a decrease in the activity of water in the medium. The relatively high temperature sensitivity of the absorbance of the titanium, vanadium, and niobium complexes is probably also a reflection of the changes in the activity of water with temperature changes. This was borne out by the observation that as the water content of the medium decreased, the change in absorbance per unit of temperature change lessened.

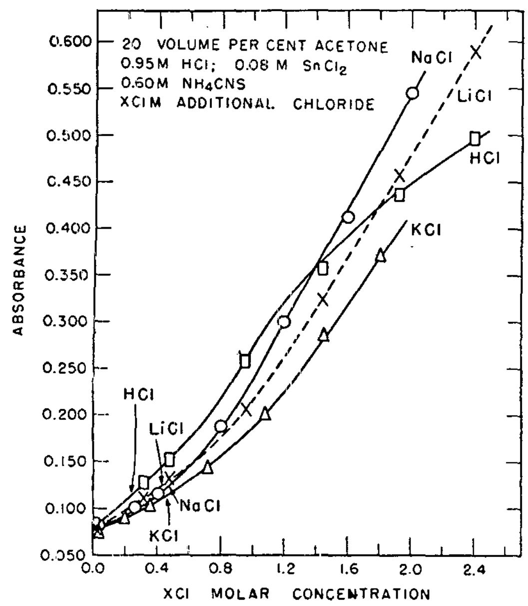

Figure 8. Effect of acid and salts on niobium color development

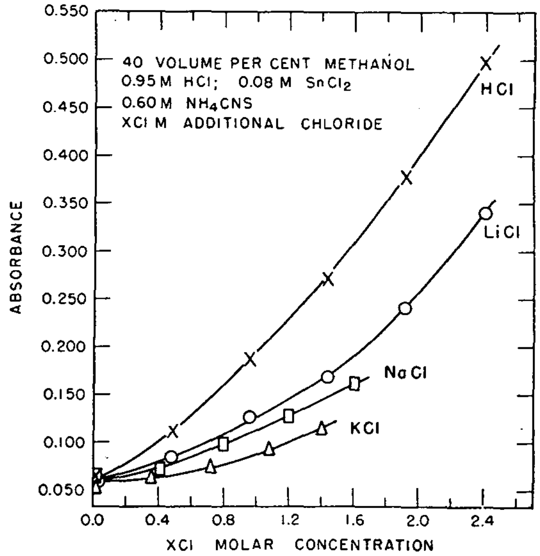

Figure 9. Effect of acid and salts on niobium color development

Table II. Distribution Coefficient of Niobium Fluoride Between Equal Volumes of Methyl Ethyl Ketone and an Aqueous Solution[a]

Sample No.	Nb^{95} Distribution, Total Counts/Min. First Equilibration Aqueous	First Equilibration Organic	First Equilibration Surface	Second Equilibration Aqueous	Second Equilibration Organic	Second Equilibration Surface	Distribution Coefficient Organic/Aqueous
1[b]	160	30400	3000	28	2500	500	190
2	150	32300	1230	20	1000	300	213
3	145	31200	2400	40	2000	490	215
4[b]	145	30400	3100	60	2500	490	209

[a] 2.8*M* HF, 3.6*M* H_2SO_4, 0.002*M* NH_4F, Nb^{95}(0.757 Mev.) tracer (Total of 43.5 µg. niobium/sample = 33650 c./m. in the well-type scintillation crystal counter).

[b] Drifilmed polyethylene container.

A number of things may complicate the observations of the effects of color development. With niobium, a reactive sample must be used to avoid slow or partial color development in the various media due to hydrolysis or polymerization of the niobium. Also the reactive niobium must be added last to the homogeneous solution and the absorbance allowed to develop to a stable value. All absorbances (Figures 8 and 9), were stable within 15 minutes, and immediately upon mixing, produced an absorbance within 10% of the stable value. In Figure 8, the absorbance development of niobium thiocyanate was studied in a standard acetone-aqueous thiocyanate medium. The base composition of the medium was selected to give relatively large absorbance changes with the salt or acid variables. The additional chloride added—such as acid, lithium, sodium, and potassium chloride—to the base solution has been plotted in Figures 8 and 9 against the absorbance at a constant niobium concentration. In Figure 8, the initial effectiveness in development of the absorbance followed the expected order of the lyotropic series hydrochloric acid > lithium chloride > sodium chloride > potassium chloride. However, the lithium chloride and hydrochloric acid did not maintain their effectiveness at higher concentrations in the acetone medium. Appreciable reaction with acetone and lithium chloride and acetone and hydrogen chloride, but not with sodium and potassium chlorides, apparently takes place. A second series shown in Figure 9 where methanol replaced acetone showed no evidence of abnormal solvent interaction with any of the chlorides, and the lyotropic series hydrochloric acid > lithium chloride > sodium chloride > potassium chloride was maintained at all concentrations observed. Repeated checks of many of the points in both Figures 8 and 9 gave very reproducible results. With media of higher acetone and acid content, the absorbance is not so sensitive to salt or acid variations (Figure 6). Sulfuric acid shown in Figure 6 will show marked bleaching effects in low acetone media, because the bivalent sulfate ion concentration becomes appreciable and complexes the niobium very effectively.

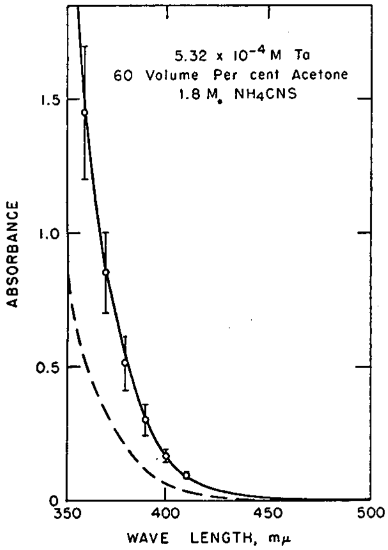

Figure 10. Tantalum thiocyanate complex

The literature on the analysis of niobium-uranium mixtures was examined in some detail. Milner and Wood (*9*) have studied the extraction of niobium fluoride from uranyl ion. The extracted niobium fluoride finally was determined gravimetrically. This extraction procedure, with slight modifications, has been applied successfully to the spectrophotometric analysis of niobium.

Table II shows the results of niobium-95 tracer studies of the extraction procedure, each extraction being made on a total of 43.5 γ of niobium. Based on analyses of both phases, the distribution coefficient is sufficiently high so that after one extraction with equal volumes of organic and aqueous solutions, only 0.5% of the original niobium should remain in the aqueous phase. However, the amount of activity remaining on the surface of the 50-ml. polyethylene vessel was surprisingly large for a fluoride solution. Thus, after two extractions in the same polyethylene vessel, slightly more than 1% of the total niobium remained on the surface. Drifilming this surface appeared to be without effect. This emphasizes the necessity for special care in handling niobium solutions of low concentrations. The total counts on the surface were obtained by difference and are known with an accuracy of approximately ±15%.

The extraction procedure was especially effective in separating niobium from nickel, iron, and uranium (Table III). Vanadium and tungsten were partially extracted with niobium.

TANTALUM

The color formation of tantalum in thiocyanate acetone medium was judged not to be analytically useful. Figure 10 shows a scan of the tantalum thiocyanate absorbance and the order of magnitude of the deviations. The errors were a result of the inability to prevent hydrolysis and/or polymerization of the tantalum.

The sample, in platinumware, was fumed with concentrated sulfuric acid or hydrofluoric-sulfuric acid mixtures to a final volume of 0.5 ml. The sample was then transferred into 15 ml. of 3*M* ammonium thiocyanate in acetone with 3 ml. of 8*M* hydrochloric acid, followed by rinsing with 15 drops of 10% stannous chloride in 6*M* hydrochloric acid and finally was diluted to 25 ml. with water. Any direct dilution of the fumed tantalum with water caused a marked decrease in the absorbance.

The dotted curve in Figure 10 shows the effect of adding 10 drops of water to the fumed tantalum in 0.5 ml. of concentrated sulfuric acid before developing the color. It was much more difficult to keep the reactive species of tantalum in solution than in the case of niobium.

The tantalum inhibited the niobium color formation only when reactive tantalum hydrolyzed in the presence of reactive niobium. Thus, if a tantalum solution were diluted with water to approximately 6 to 7*M* sulfuric acid and then mixed with niobium, the inhibiting effect on the niobium color development was not evident. Also it was possible to apply the procedure to mixtures of tantalum and niobium in sulfuric acid (1 to 5 parts of tantalum and 1 part of niobium) and develop the color without interference. However, extreme care had to be exercised to prevent hydrolysis before complexing. Tantalum(V) and niobium(V) mixtures were oxidized to peroxy compounds in fuming sulfuric acid with a few drops of peroxide. The peroxy compounds of tantalum(V) and niobium(V) were then added to the thiocyanate-acetone medium and reduced to the quinquevalent compounds in the color developing solution with the stannous chloride. Although this technique was not completely trouble free, a large number of the niobium(V) thiocyanate absorbances developed correctly in the presence of reactive tantalum (> 75%), where failure was almost certain previously.

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Received for review September 23, 1954. Accepted December 13, 1954.

X-Ray Diffraction Powder Data for Steroids

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X-ray diffraction powder data and powder diffraction photographs for 27 steroids are presented. Visual comparison of the powder patterns for known and unknown steroids was found to be sufficient for identification.

LARGE numbers of well crystallized organic compounds exist which give x-ray diffraction patterns satisfactory as a means of identification when their pattern data are available. Most of the steroid group of compounds fall into this category. Bernal, Crowfoot, and Fankuchen (*1*) published crystallographic and optical data for some 80 sterol derivatives but did not include powder diffraction data. The purpose of this paper is to present powder data for 27 steroids.

PROCEDURE

The melting point of each compound (Table I) was determined and checked with the literature to be certain of the purity and identity of these steroids. The crystals were powdered in a

Table I. Index to Steroid X-Ray Diffraction Powder Data

Pattern No.	Name	Melting Point (Uncorr.), ° C.
	C_{18} STEROIDS	
1	Estriol	280–282[a]
2	Estrone	259–261
3	Estradiol	176–179
	C_{19-29} STEROIDS	
	A. Monohydric Alcohols	
A1	Stigmasta-Δ5,22-dien-3(β)-ol (stigmasterol)	167–169
A2	Δ^5-Cholesten-3(β)-ol (cholesterol)	146–148
A3	Cholestan-3(β)-ol (dihydrocholesterol)	141–142
A4	Δ^5-Stigmasten-3(β)-ol (β sitosterol)	138–140
	B. Dihydric Alcohols	
B1	Δ^5-Androstene-3(β),17(β)-diol	180–182
B2	Δ^5-Androstene-3(β),17(β)-diol-17-benzoate	218–220
	C. Diketones	
C1	Δ^4-Androstene-3,17-dione	172–174
C2	Δ^4-Pregnene-3,20-dione (progesterone)	120–122
	D. Monohydroxy-Monoketones	
D1	Δ^5-Androstene-3(β)-ol-17-one (dehydroepiandrosterone)	151–153
D2	Δ^5-Pregnene-3(β)-ol-20-one (pregnenolone)	191–193
D3	Δ^4-Androstene-17(α)-ol-3-one (testosterone)	152–155
D4	Δ^5-Androstene-3(β)-ol-17-one-3-acetate (dehydroepiandrosterone acetate)	168–170
D5	Androstan-3(α)-ol-17-one (androsterone)	184–185
D6	$\Delta^{5,16}$-Pregnadiene-3(β)-ol-20-one (dehydropregnenolone)	173–175
D7	16,17-Epoxy-Δ^5-pregnene-3(β)-ol-20-one-3-acetate (epoxypregnenolone acetate)	155–158
D8	Δ^5-Pregnene-3(β)-ol-20-one-3-acetate (pregnenolone acetate)	149–151
D9	17(β)-methyl-Δ^4-androstene-17(α)-ol-3-one (methyltestosterone)	161–164
D10	Δ^5-Pregnene-3(β)-ol-20-one-3-methyl ether (pregnenolone methyl ether)	125–127
D11	Δ^5-Pregnene-3(β),21-diol-20-one-21-acetate (acetoxypregnenolone)	183–185
	E. Monohydroxy-Monocarboxyacids	
E1	3(β)-Hydroxy-bisnor-Δ^5-cholenic acid	291–293[b]
E2	3(β)-Acetoxy-bisnor-Δ^5-cholenic acid	234–236
	BILE ACIDS	
F1	Cholic acid	196–198
F2	Desoxycholic acid	171–173
F3	Dehydrocholic acid	234–236

[a] 4° rise per minute.
[b] Decomposed.

Table II. Powder Diffraction Data for Steroids

C_{18} STEROIDS

d, A.	I/I_1	d, A.	I/I_1	d, A.	I/I_1
1. Estriol		2. Estrone[a]		3. Estradiol	
8.08	3	9.36	2	7.50	4
6.73	8	7.26	2	6.71	9
6.05	5	5.56	2	6.03	4
4.81	3	6.19	6	5.64	10
4.34	10	5.85	9	5.00	4
4.16	4	5.13	7	4.78	9
3.36	3	4.85	8	4.63	4
2.85	2	4.40	10	4.32	6
2.47	2	4.01	6	4.08	4
2.28	2	3.85	6	3.92	6
2.11	1	3.70	5	3.72	5
1.88	3	3.59	1	3.35	6
		3.27	6	3.24	3
		3.18	1	3.13	3
		3.14	5	3.02	3
		3.01	1	2.90	2
		2.94	5	2.80	4
		2.74	5	2.65	1
		2.57	6	2.56	2
		2.44	4	2.49	4
		2.43	4	2.40	2
		2.36	4	2.32	1
		2.23	6	2.27	2
		2.17	2	2.21	2
		2.10	2	2.14	4
		2.06	2	2.08	2
		2.01	6	2.00	2
		1.95	2	1.93	3
		1.92	2	1.86	2
		1.88	1	1.79	2
		1.79	1		
		1.74	1		
		1.70	1		
		1.65	1		

C_{19-29} STEROIDS

A. Monohydric Alcohols

d, A.	I/I_1	d, A.	I/I_1	d, A.	I/I_1	d, A.	I/I_1
A1. Stigmasta-$\Delta^{5,22}$-dien-3(β)-ol		A2. Δ^5-Cholesten-3(β)-ol		A3. Cholestan-3(β)-ol		A4. Δ^5-Stigmasten-3(β)-ol	
12.9	6	17.0	3	17.0	7	13.2	1
9.21	4	13.4	4	13.6	5	9.36	1
7.87	7	11.4	3	11.3	6	7.41	6
6.69	8	9.26	3	9.76	2	7.00	4
6.30	2	8.43	7	8.76	4	5.91	10
6.01	8	7.76	2	7.31	4	4.81	9
5.77	6	6.86	6	6.71	6	4.61	7
5.50	1	6.26	6	6.21	7	4.05	7
5.13	10	5.74	9	5.59	9	3.48	5
4.75	5	5.26	10	5.17	10	3.27	2
4.47	6	4.91	8	4.76	5	2.92	1
4.25	4	4.58	5	4.52	3	2.83	1
4.05	3	4.22	5	4.33	3	2.43	1
3.88	5	3.97	1	4.10	5	2.28	2
3.45	4	3.80	4			1.19	1
		3.58	3				
		3.41	2				
		3.19	2				
		2.94	1				
		2.66	1				
		2.42	1				
		2.27	2				
		2.22	1				
		2.14	3				

B. Dihydric Alcohols

d, A.	I/I_1	d, A.	I/I_1	d, A.	I/I_1	d, A.	I/I_1
B1. Δ^5-Androstene-3(β),17(α)-diol				B2. Δ^5-Androstene-3(β),17(β)-diol-17 benzoate			
10.8	6	3.11	5	14.9	2	2.92	2
8.47	7	3.02	5	8.31	3	2.83	2
7.09	1	2.94	7	7.38	2	2.78	2
6.05	7	2.82	5	6.05	10	2.72	1
5.87	10	2.71	6	5.59	4	2.59	1
5.58	10	2.58	5	5.32	10	2.50	1
5.39	7	2.56	4	5.02	1	2.39	2
5.00	8	2.48	4	4.67	7	2.32	3
4.82	6	2.38	5	4.50	4	2.22	2
4.50	2	2.31	6	4.30	7	2.13	2
4.28	8	2.10	6	3.92	6	2.09	2
4.09	7	2.05	5	3.69	6	2.03	2
3.85	6	1.98	5	3.59	6	1.96	1
3.72	6	1.94	1	3.47	6		
3.60	2	1.87	1	3.35	2		
3.46	2	1.78	1	3.23	2		
3.38	7	1.73	1	3.14	2		
3.27	5			3.05	2		

[a] Six-sided plates.

(*Continued on page 516*)

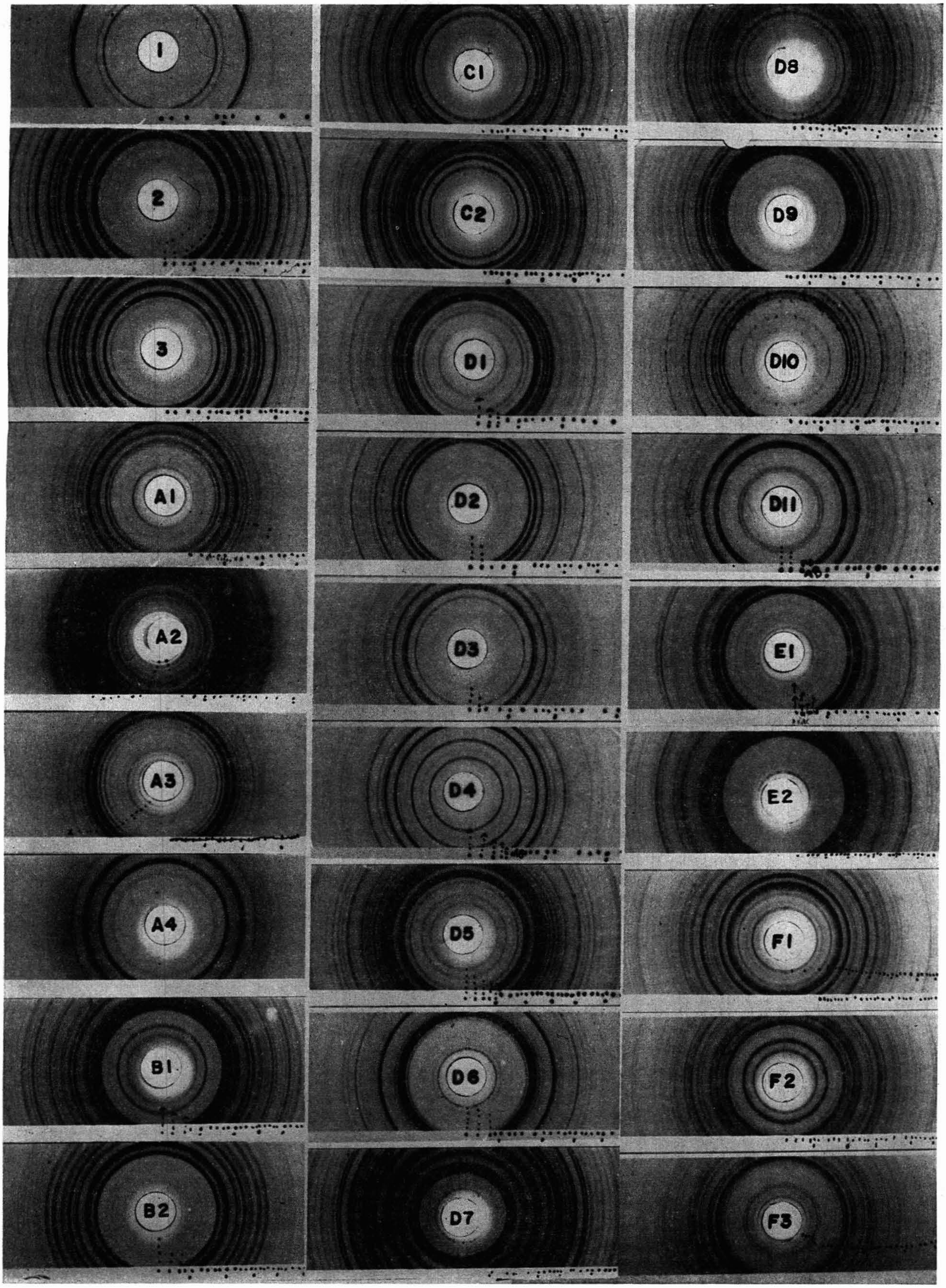

Figure 1. X-ray diffraction powder patterns of steroids

Key found in Table I

Table II. Powder Diffraction Data for Steroids (*Continued*)

C_{19-29} STEROIDS (*Continued*)

C. Diketones

d, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1
C1. Δ^4-Androstene-3,17-dione				C2. Δ^4-Pregnene-3,20-dione (β)			
10.4	1	2.98	5	9.33	3	3.08	2
8.51	1	2.80	6	8.33	8	3.01	3
7.09	7	2.69	1	6.96	9	2.90	2
6.71	1	2.51	6	6.12	7	2.78	3
6.01	10	2.43	5	5.73	7	2.69	3
5.57	7	2.38	3	5.24	10	2.60	2
5.16	9	2.31	4	4.83	7	2.39	2
4.68	2	2.24	4	4.64	4	2.32	3
4.45	3	2.12	5	4.51	4	2.26	3
4.24	8	2.08	2	4.19	5	2.22	2
4.00	3	2.03	4	3.95	7	2.16	2
3.66	4	2.00	4	3.71	3	2.11	2
3.53	7	1.95	3	3.46	6	2.04	3
3.19	6			3.36	7	1.97	2
3.13	2			3.24	1	1.86	2
3.05	2			3.16	1	1.83	3

D. Monohydroxy-Monoketones

d, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1
D1. Δ^5-Androstene-3(β)-ol-17-one (Leaflets)		D2. Δ^5-Pregnene-3(β)-ol-20-one		D3. Δ^4-Androstene-17(α)-ol-3-one	
10.9	5	12.1	5	10.2	4
7.53	6	8.47	5	7.56	4
6.78	5	5.97	10	6.84	8
5.95	10	5.47	10	5.77	10
5.72	10	4.95	8	5.54	8
5.29	7	4.30	6	4.84	9
5.00	8	4.02	9	4.36	7
4.71	9	3.83	6	4.04	1
4.22	4	3.47	1	3.87	1
3.94	2	3.35	1	3.74	2
3.60	3	3.20	9	3.48	4
3.33	4	2.92	7	3.26	4
3.05	7	2.83	4	3.01	4
2.82	1	2.74	1	2.59	4
2.51	1	2.66	1	2.41	3
2.20	2	2.59	1	2.18	3
		2.37	2	2.02	2
		2.23	2		
		1.99	6		
		1.87	1		
		1.80	1		
		1.72	1		

d, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1
D4. Δ^5-Androstene-3(β)-ol-17-one-3-acetate		D5. Androstan-3(α)-ol-17-one		D6. $\Delta^{5,16}$-Pregnadiene 3(β)-ol-20-one	
9.99	8	11.1	4	12.5	8
8.31	3	8.67	4	10.6	3
6.73	8	7.38	2	8.12	1
6.05	3	6.81	4	6.13	4
5.49	3	6.46	2	5.74	10
5.17	10	5.91	10	5.44	10
4.99	2	5.11	7	5.01	4
4.70	2	4.80	8	4.83	2
4.54	10	4.63	6	4.22	9
4.41	6	4.52	6	4.10	9
4.12	6	4.28	5	3.93	1
3.72	7	4.12	5	3.81	1
3.58	4	3.94	4	3.47	2
2.95	5	3.79	4	3.30	7
2.74	4	3.63	5	3.18	2
2.62	4	3.51	1	3.08	3
2.47	4	3.30	1	2.97	3
2.28	1	3.19	6	2.73	1
2.14	1	3.06	4	2.02	1
		2.96	4	1.88	1
		2.84	5		
		2.70	4		
		2.54	1		
		2.48	1		
		2.42	1		
		2.33	4		
		2.25	3		
		2.20	3		
		2.15	6		
		1.97	5		
		1.91	5		
		1.79	1		

d, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1
D7. 16,17-Epoxy-Δ^5-pregnene-3(β)-ol-20-one-3-acetate				D8. Δ^5-Pregnene-3(β)-ol-20-one-3-acetate			
13.1	3	2.62	2	8.15	6	2.84	3
8.04	9	2.50	2	7.66	3	2.71	3
7.53	5	2.41	2	7.00	9	2.66	1
7.09	5	2.35	3	6.61	2	2.54	1
5.79	8	2.28	3	6.28	8	2.48	2
5.01	8	2.21	2	6.06	8	2.41	2

D. Monohydroxy-Monoketones (*Continued*)

d, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1
D7. 16,17-Epoxy-Δ^5-pregnene-3(β)-ol-20-one-3-acetate (*continued*)				D8. Δ^5-Pregnene-3(β)-ol-20-one-3-acetate (*continued*)			
4.82	10	2.19	2	5.74	10	2.29	3
4.68	3	2.12	1	5.50	10	2.23	3
4.16	5	2.08	1	5.16	3	2.17	3
3.87	4	2.02	4	4.75	10	2.07	1
3.70	7	1.95	1	4.47	6	2.04	1
3.54	2	1.90	3	4.26	4	2.01	2
3.41	3	1.85	2	4.02	4	1.96	4
3.30	2	1.77	2	3.82	4	1.88	3
3.23	6	1.62	2	3.63	10	1.84	3
3.00	4	1.57	1	3.41	4		
2.94	3	1.54	1	3.29	3		
2.85	2	1.49	2	3.21	3		
2.81	2	1.32	2	3.11	4		
2.71	1	1.23	1	2.97	3		

d, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1
D9. 17(β)-Methyl-Δ^4-androstene-17(α)-ol-3-one		D10. Δ^5-Pregnene-3(β)--ol-20-one-3 methyl ether		D11. Δ^5-Pregnene-3(β),21-diol-20-one-21 acetate	
12.3	3	11.9	5	9.77	7
10.8	3	8.39	6	9.03	7
9.56	3	6.49	1	6.66	3
7.06	3	6.07	10	6.37	3
6.09	8	5.74	10	5.97	10
5.87	3	5.41	4	5.72	10
5.64	10	5.10	9	5.17	3
5.31	9	4.71	9	4.85	10
5.06	5	4.43	4	4.28	4
4.76	5	4.22	3	4.15	4
4.48	7	3.92	9	3.97	4
4.18	6	3.82	2	3.66	4
3.82	4	3.70	2	3.51	2
3.67	6	3.43	2	3.29	4
3.46	4	3.30	3	3.00	4
3.24	4	3.18	7	2.82	2
3.06	4	3.05	3	2.69	3
2.98	4	2.92	3	2.56	3
2.86	3	2.74	3	2.38	2
2.75	1	2.71	3	2.36	2
2.53	5	2.63	3	2.20	3
2.43	1	2.50	1	2.09	2
2.38	1	2.39	8	1.98	2
2.25	1	2.25	2	1.89	1
2.21	1	2.19	2		
2.04	1	2.10	1		
1.99	1	2.00	2		
1.85	1	1.96	2		
1.78	1	1.80	1		

E. Monohydroxy-Monocarboxyacids

d, A.	I/I_1	*d*, A.	I/I_1
E1. 3(β)-Hydroxy-bisnor-Δ^5-cholenic acid		E2. 3(β)-Acetoxy-bisnor-Δ^5-cholenic acid	
13.7	3	14.4	5
8.85	2	5.97	10
7.26	2	5.75	5
5.91	9	5.55	9
5.25	10	5.37	6
5.03	7	5.08	10
4.66	5	4.82	9
4.15	5	4.68	7
4.02	5	4.53	6
3.76	3	4.43	4
3.53	2	4.22	1
3.35	3	4.01	1
3.27	3	3.81	4
3.12	2	3.72	7
3.01	7	3.53	3
2.92	6	3.39	4
2.69	5	3.32	1
2.48	1	3.14	5
2.39	1	3.08	4
2.31	2	3.01	3
2.14	2	2.96	7
2.07	1	2.86	1
1.98	2	2.78	1
		2.68	1
		2.62	3
		2.55	4
		2.47	2
		2.38	4
		2.28	3
		2.21	3
		2.13	2
		2.06	2
		2.00	3
		1.91	3
		1.82	1
		1.74	1

Table II. Powder Diffraction Data for Steroids (*Continued*)

d, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1
		F. Bile Acids			
F1. Cholic Acid		F2. Desoxycholic Acid		F3. Dehydrocholic Acid	
11.4	6	12.0	6	15.1	5
9.83	3	8.98	8	12.9	4
7.94	6	6.92	2	11.9	2
7.06	2	6.28	10	10.3	5
6.78	9	5.87	3	9.61	4
6.49	9	5.66	3	7.83	1
6.19	1	5.41	7	6.86	1
5.93	1	5.19	3	6.39	4
5.74	10	5.03	6	5.93	5
5.37	2	4.47	3	5.66	10
5.16	3	4.27	4	5.26	7
4.94	2	3.97	6	4.96	7
4.75	3	3.85	4	4.76	1
4.45	8	3.69	5	4.62	3
4.24	2	3.41	3	4.47	6
4.00	5	3.33	3	4.25	5
3.82	2	3.24	4	4.15	6
3.69	4	3.07	2	3.97	4
3.56	4	2.88	1	3.84	2
3.43	1	2.77	2	3.63	2
3.31	2	2.71	1	3.45	4
3.19	3	2.61	3	3.32	3
3.11	1	2.52	3	3.16	4
3.02	1	2.46	4	3.03	3
2.87	1	2.40	1	2.95	3
2.72	2	2.36	1	2.67	2
2.64	1	2.32	2	2.65	2
2.56	4	2.13	1	2.57	2
2.51	2	2.00	2	2.46	2
2.46	2	1.94	2	2.41	1
2.41	2	1.83	2	2.35	2
2.35	2			2.29	1
2.24	2			2.23	2
2.19	1			2.13	2
2.13	2			2.07	2
2.06	1				
1.99	1				

mortar to a fineness of about 200 mesh. The powders were placed in thin wall plastic tubes of 0.01-mm. wall thickness and 0.2-mm. diameter (*2*, *3*). The mounted samples were exposed to nicke filtered copper radiation produced at 35 KVP and 20-ma. current for 5 hours in Norelco (North American Philips Co., Inc., Mount Vernon, N. Y.) of 114.59-mm. diameter, Debye-Scherrer cameras. The patterns (Figure 1) were recorded on Kodak No-Screen x-ray film, developed for 5 minutes at 68° F. in Kodak x-ray developer. Relative intensities (I/I_1) of the pattern lines (Table II) were determined by visual estimation with the strongest line noted as 10. The *d* values are given in Angstrom units with the copper radiation weighted wave-length value of 1.5418 A. used as the basis of spacing calculations.

The wider use of the x-ray method of analysis for organic compounds should supplement melting point determinations and the use of infrared absorption analysis (*4*). A distinct advantage of this method is that very small quantities of sample are required—one milligram is usually sufficient for an analysis. Visual comparison with standard diffraction patterns usually (Figure 1) has been found sufficient for identification of these steroids, without resort to *d* value calculation.

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Received for review September 16. 1954. Accepted December 6, 1954.

Infrared Spectra of Alkyl Hydroperoxides

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The infrared spectra of 17 alkyl hydroperoxides, both primary and secondary, have been recorded between 2 and 15 microns. In each case the hydroperoxide showed a characteristic but rather weak absorption in the region 11.4 to 11.8 microns, which has been previously correlated with the oxygen-oxygen stretching vibration. This is a region in which some of the corresponding alcohols also absorb and thus it is not a unique means of characterizing the hydroperoxide. All primary and secondary alkyl hydroperoxides showed absorption in the 12.0- to 12.5-micron region. A most interesting alternating pattern is shown by normal alkyl hydroperoxides in this region; those which had an even number of carbon atoms (butyl through decyl) showed two maxima (at about 12.1 and 12.4 microns) while the odd-numbered members of the series showed a single maximum at about 12.3 microns.

STUDY of the infrared spectra of hydroperoxides has been hindered by lack of sufficient examples of pure members of this class. The availability of many new primary and secondary alkyl hydroperoxides (*9*) has permitted a more extensive study than was previously possible (*3*, *4*, *7*). In the present study the infrared spectra of nine primary and eight secondary pure liquid alkyl hydroperoxides (*9*) were recorded in the range 2 to 15 microns. The spectra of the corresponding alcohols were taken in each case for comparison. The important features of these spectra are recorded in Tables I to IV and Figures 1 to 7.

EXPERIMENTAL

Materials. The spectra were taken in each case on analytically pure (determined by combustion) alkyl hydroperoxides made by the methods outlined previously (*9*). The samples were used soon after preparation and were stored in the dark and cold until ready for use. The determinations were made without solvent using both a 0.003-inch polyethylene spacer and a thinner film which resulted when no spacer was employed. The spectra for both thicknesses are represented in Figures 1, 2, 3, and 4.

Spectral Determinations. All spectra were determined on the Perkin-Elmer double beam recording Model 21 infrared spectro-

Table I. Wave Length of OH Stretching Band of Alkyl Hydroperoxides and Corresponding Alcohols

R	R-OH, μ	R-OOH, μ	Shift, μ
n-Propyl	2.98	2.94	0.04
n-Butyl	2.99	2.96	0.03
Isoamyl	2.99	2.95	0.04
n-Amyl	2.99	2.96	0.03
n-Hexyl	3.00	2.94	0.06
n-Heptyl	3.01	2.92	0.09
n-Octyl	3.00	2.96	0.04
n-Nonyl	3.02	2.97	0.05
n-Decyl	3.00	2.98	0.02
sec-Butyl	2.99	2.96	0.03
Cyclopentyl	2.98	2.95	0.03
2-Pentyl	3.00	2.96	0.04
3-Pentyl	2.95	2.90	0.05
2-Hexyl	2.96	2.93	0.03
3-Hexyl	2.98	2.95	0.03
2-Heptyl	2.99	2.98	0.01
2-Octyl	2.96	2.91	0.05
t-Butyl	2.96	2.91	0.05

photometer equipped with a rock salt prism. The instrument was operated at the following settings: resolution 927; response, 1:1; gain, 6; suppression, 1.

In several cases there was occasion to retake the spectra of a particular hydroperoxide (in two cases on a sample which had been stored for 10 months); in all these cases the spectra were completely reproducible.

DISCUSSION

The absorption in the region of 2 to 5.6 microns for the 16 hydroperoxides was almost identical for both the alcohol and the corresponding hydroperoxide. In every case, however, the O—H stretching band of the hydroperoxide appeared at a slightly shorter wave length than that for the corresponding alcohol, indicating a slight but appreciably greater hydrogen bonding in the pure liquid state for the alcohol. Milas and Mageli (*1*) found the reverse shift in several acetylenic hydroperoxides. The wave lengths of the O—H stretching band for both the hydroperoxide and the corresponding alcohol are compiled in Table I. In these compounds the only difference in structure is the replacement of the —OH group with the —OOH group. The spectra of *tert*-butyl hydroperoxide (*7*) are added to this and subsequent tables for purposes of comparison.

All the hydroperoxides and alcohols absorbed moderately strongly near 6.80 and 7.2 microns, regions assigned to the deformation vibration of the CH$_2$— group (*5*). In addition, all of the primary hydroperoxides showed characteristic weak absorption maxima near 6.72 and 6.98 microns—that is, this region was a singlet for the primary alcohol (column D, Table II) but a triplet for the primary hydroperoxide (columns A, B, and C, Table II). As the homologous series was ascended these maxima near 6.72 and 6.98 microns became progressively less discrete until in the case of *n*-decyl, these were inflections on the main 6.80-micron band. This is shown in Figure 5. It seems likely that secondary bands which are present in the hydroperoxide but absent in the alcohol result from interactions of the hydrogen of the alpha methylene group with the unsymmetrical hydroperoxy radical. The secondary hydroperoxides and alcohols also absorbed at or near 6.80 microns, but none exhibited the characteristic triplet absorption of the primary hydroperoxides. Accordingly this appears to be a definitive, although not very prominent, feature of the infrared spectra of the saturated primary alkyl hydroperoxides studied.

All of the spectra, except those of cyclopentyl hydroperoxide and cyclopentanol, showed absorption at or near 7.20 microns (columns E and G, Table II) which has been attributed to the wagging or rocking vibration of the CH$_3$— group (*5*). In addition to this major band, all of the secondary hydroperoxides showed a somewhat weaker band at 7.40 to 7.50 microns (column F, Table II, Figure 6). This absorption was absent in the spectra of the primary hydroperoxide and thus this is a definitive characteristic of those secondary alkyl hydroperoxide studies.

Table II. Absorption of Alkyl Hydroperoxides and Alcohols in the Region 6.7 to 7.5 Microns

Alkyl Group	Hydroperoxides A[a]	B	C[a]	Alcohol D	Hydroperoxide E	F[b]	G	Alcohol H[b]
n-Propyl	6.72	6.82	6.93	6.83	7.20	...	7.20	...
n-Butyl	6.72	6.81	6.96	6.82	7.24	...	7.24	...
n-Amyl	6.72	6.80	6.96	6.80	7.23	...	7.23	...
Isoamyl	6.72	6.80	6.96	6.80, 7.0	7.20	7.28	7.20	7.28
n-Hexyl	6.72	6.80	6.96	6.82	7.22	...	7.24	7.44[c]
n-Heptyl	6.72	6.80	6.96	6.81	7.23	...	7.24	7.44[c]
n-Octyl	6.74	6.81	6.96	6.82	7.24	...	7.24	...
n-Nonyl	6.74	6.82	6.96	6.84	7.25	...	7.25	...
n-Decyl	6.74[b]	6.82	6.96[b]	6.81	7.24	...	7.24	7.44[c]
2-Butyl	...	6.83	...	6.84	7.24	7.49	7.26	7.54
Cyclopentyl	...	6.94	...	6.86	...	7.42	...	7.42
2-Pentyl	...	6.83	...	6.84	7.24	7.41	7.25	...
3-Pentyl	...	6.81	...	6.82	7.20	7.39	7.23	7.44
2-Hexyl	...	6.81	...	6.82	7.24	7.48	7.24	7.52
3-Hexyl	...	6.82	...	6.82	7.22	7.38	7.22	7.52
2-Heptyl	...	6.84	...	6.84	7.25	7.50	7.25	...
2-Octyl	...	6.80	...	6.80	7.23	7.46	7.24	...
t-Butyl	6.75	6.80	...	6.76	7.29	...	7.22	7.30

[a] In almost every case these secondary bands in the triplet, columns A and C, or the doublet, column F, were weak.

[b] These points were inflections only. These bands became progressively less discrete as homologous series was ascended.

[c] Very weak and scarcely discernible.

The absorption of cumene hydroperoxide at 9 to 10 microns has been attributed to the isopropyl group. As seen in Table III, both the alcohols and hydroperoxides absorb in this region and there does not appear to be any particular absorption band in this region which is uniquely characteristic of any one of the classes of compounds studied. Absorption at 9.26 to 9.71 microns has been ascribed to the deformation frequency of the OH group (*2*).

Alcohols absorb in the region between 8 and 10 microns and Zeiss and Tsutsui (*10*) have assigned a wave length of 9.30 to 9.45 microns to the carbon-oxygen stretching vibration of the saturated normal primary alcohols with the exception of methyl alcohol which absorbs at 9.7 microns in this region. A similar assignment of 8.95 to 9.03 microns for the simple unbranched saturated secondary alcohols was made. Branching, unsaturation on the alpha carbon, or an aromatic ring on the alpha carbon atom, was shown by Zeiss and Tsutsui to cause considerable shift of this characteristic band.

Table III. Absorption Spectra in the Region of 8.7 to 9.7 Microns for Alkyl Hydroperoxides and Corresponding Alcohols

Alkyl Group	Alkyl Hydroperoxide Wave Length, μ A	B	C	D[a]	Alcohol Wave Length, μ E	F[b] (*9*)
n-Propyl	8.92	9.44	9.60	9.08	9.32, 9.44	9.45
n-Butyl	8.86	9.40	9.76	8.98	9.32	9.30
n-Amyl	8.84	9.53	9.72	8.94	9.46	9.45
Isoamyl	8.75	9.52	9.88	8.88	9.42	9.45
n-Hexyl	8.84	9.50	9.86	8.94	9.44	9.45
n-Heptyl	8.86	9.46	9.72	8.94	9.44	[c]
n-Octyl	8.86	9.64	9.88	8.94	9.46	9.45
n-Nonyl	8.87	9.56	9.88	8.93	9.46	9.45
n-Decyl	8.88	9.64	...	8.90	9.46	[c]
2-Butyl	8.80–9.00[d]	9.72	...	8.62–9.02[d]	9.69	9.05[a]
2-Pentyl	8.80–8.94[d]	9.42	9.70	8.72–8.98[d]	9.66	9.04[a]
3-Pentyl	8.80–8.92[d]	9.66	...	8.70–8.88, 9.0[c]	9.60	9.04[a]
Cyclopentyl	... ...	9.66	...	... ...	9.33, 9.64	9.40[a]
2-Hexyl	8.72–8.96[d]	9.48	9.82	8.71–8.99[d]	9.48, 9.80	9.04[a]
3-Hexyl	8.80–8.92[d]	9.40	9.64	8.74–8.92[d]	9.38, 9.62	9.03[a]
2-Heptyl	8.74–8.96[d]	9.40	9.70	8.74–8.98[d]	9.43, 9.70	9.03[a]
2-Octyl	8.70–8.93[d]	9.32	9.60	8.72–8.96[d]	9.34, 9.60	9.02[a]
tert-Butyl	... ...	...	9.68	8.30 ...	9.76 ...	8.30

[a] Values for maxima obtained for secondary alcohols by Zeiss and Tsutsui (column F) do not coincide exactly with any reported here. Presumably they are the second peak of the doublet reported in column D with exception of cyclopentanol.

[b] Column F reports values recorded in (*10*); Zeiss and Tsutsui (*10*) have assigned this band to C—O stretching vibration of alcohols.

[c] Not reported in (*10*).

[d] Bands reported by a dash are closely associated as a doublet.

Table IV. Infrared Absorption by Alkyl Hydroperoxides in Region of 11.4 to 11.8 Microns

Alkyl Group	Alkyl Hydroperoxide Wave Length, μ	Alcohol Conflicting Absorption Wave Length, μ
n-Propyl	11.57	11.64
n-Butyl	11.73	11.79
Isoamyl	11.52	
n-Amyl	11.64	
n-Hexyl	11.52	
n-Heptyl	11.67, 11.82	11.52
n-Octyl	11.64	
n-Nonyl	11.64	11.40
n-Decyl	11.66	
2-Butyl	11.66	
Cyclopentyl	11.50 (weak)	11.86
2-Pentyl	11.58	11.70
3-Pentyl	11.55	11.68
2-Hexyl	11.48	11.85
3-Hexyl	11.54	11.86
2-Heptyl	11.75	
2-Octyl	11.53	11.86
tert-Butyl	11.25 (weak) 11.82 (strong)	

This region of the spectra of the primary and secondary saturated alkyl hydroperoxides is tabulated in Table III. It is seen that the band at 9.32 to 9.46 microns for the normal primary alcohols has been more or less uniformly shifted to longer wave lengths (about 10 microns on the average) in the corresponding hydroperoxide. In general, the band broadens as the molecular weight of the alkyl hydroperoxide increased.

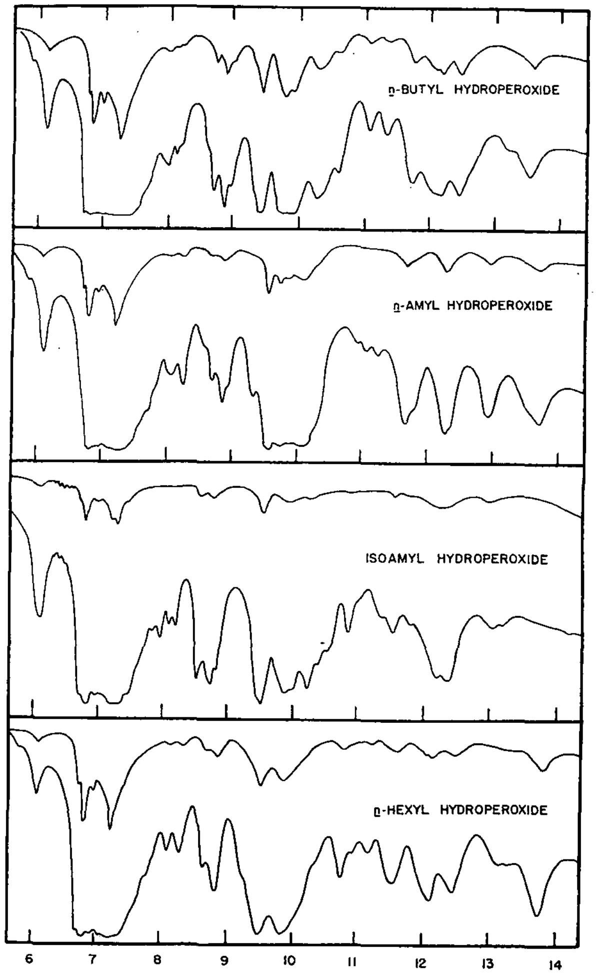

Figure 1. Infrared spectra of *n*-alkyl hydroperoxides, 6 to 14 microns

The top curve in each case was taken on a thin film without spacer while the bottom curve was taken with a 0.003-inch spacer

In the case of the secondary alkyl hydroperoxides the picture is not nearly so clear-cut. Zeiss and Tsutsui (*10*) designate a band at 9.02 to 9.05 microns as characteristic of the carbon-oxygen stretching band in unbranched secondary alcohols; their figures are given in column F in Table III. The authors did not record this exact band but presume that the one found at 8.88 to 9.02 microns was the same as reported by these workers. Not as much care was taken with the purification of the alcohols as with the purification of the hydroperoxides, nevertheless the alcohols were essentially pure and these differences are in excess of experimental error. It will be noted that both the alcohols and alkyl hydroperoxides absorb in the region 8.75 to 9.0 microns (column D, Table III), with the exception of the cyclopentyl derivatives, and the region is not therefore very definitive for the secondary alkyl hydroperoxides. Considerable study and additional examples would be necessary before definite assignments could be made with any certainty in this region; accordingly, the values for the maxima in this region are recorded in Table III and in Figures 1, 2, 3, and 4 without further speculation.

All of these saturated alkyl hydroperoxides absorbed in the region of 11.4 to 11.8 microns. Shreve (*7*) reported absorption in this region as resulting from vibration within the OOH group. The maxima in this region which are weak to medium in intensity are reported for the hydroperoxides in Table IV. Several of the alcohols also absorbed in this region and thus this is not an ideal

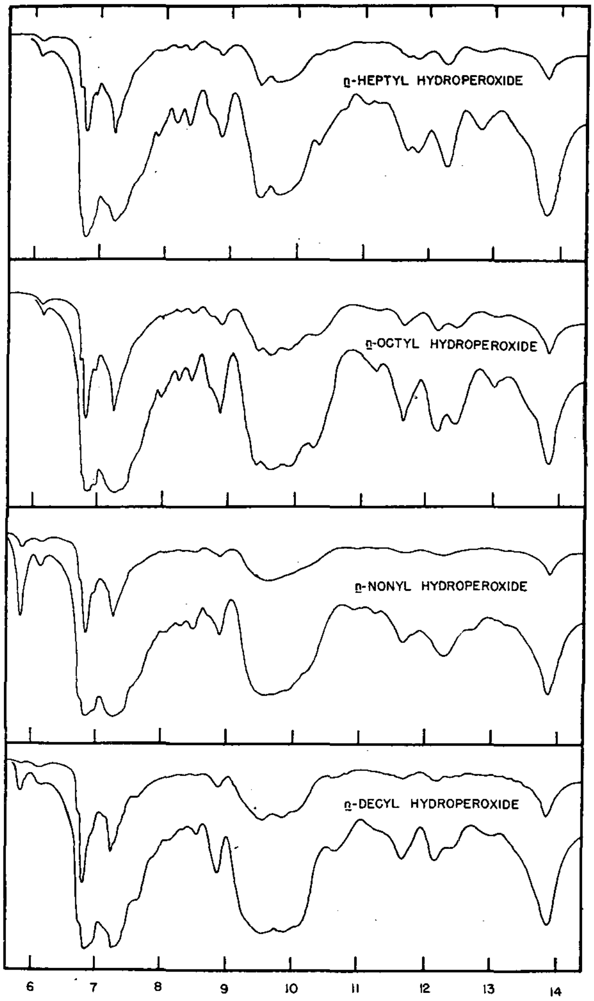

Figure 2. Infrared spectra of *n*-alkyl hydroperoxides, 6 to 14 microns

The top curve in each case was taken on a thin film without spacer while the bottom curve was taken with a 0.003-inch spacer

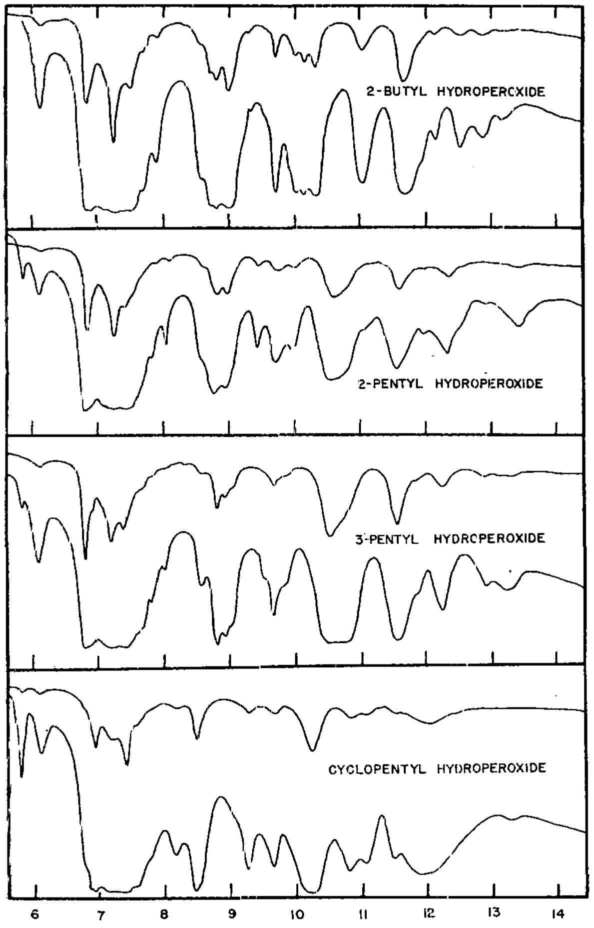

Figure 3. Infrared spectra of *sec*-alkyl hydroperoxides, 6 to 14 microns

The top curve in each case was taken on a thin film without spacer while the bottom curve was taken with a 0.003-inch spacer

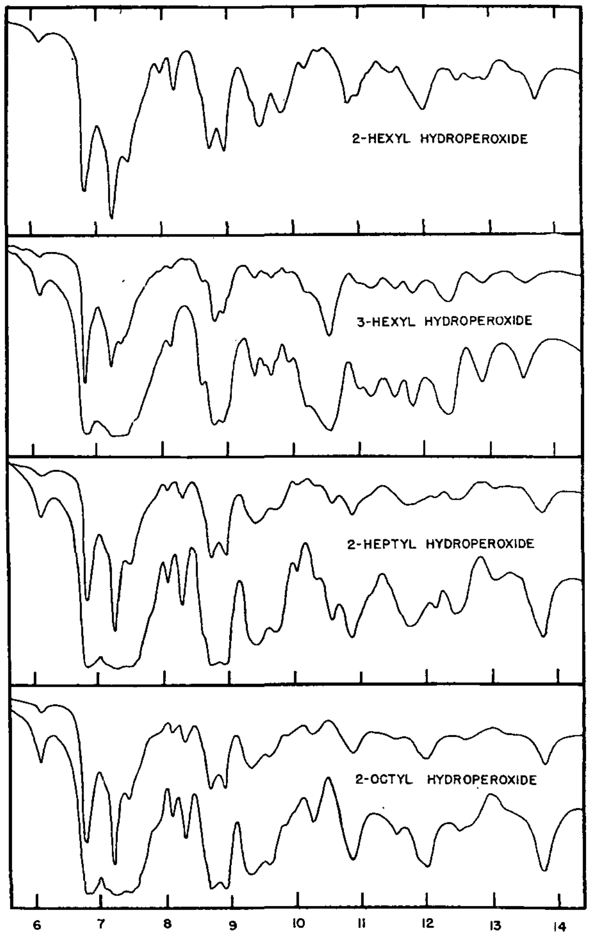

Figure 4. Infrared spectra of *sec*-alkyl hydroperoxides, 6 to 14 microns

The top curve in each case was taken on a thin film without spacer while the bottom curve was taken with a 0.003-inch spacer. The single curve for 2-hexyl hydroperoxide is with the 0.003 inch spacer

method of characterization. In every case, however, the absorption curve of the hydroperoxide differed greatly from the corresponding alcohol and could serve as a reliable method for distinguishing between the two. Table IV lists these characteristic bands for the hydroperoxides in the region 11.4 to 11.8 microns along with any conflicting absorption bands for the corresponding alcohol in this same region.

All hydroperoxides showed absorption maxima in the region of 12.0 to 12.5 microns (Table V); in the case of the *n*-alkyl hydroperoxides an extremely interesting alternating pattern was apparent as

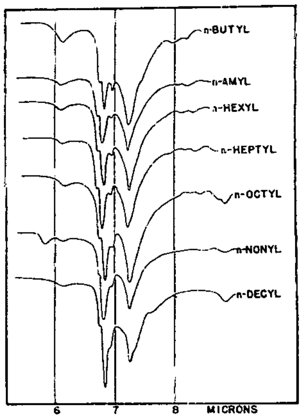

Figure 5. Comparison of infrared spectra of the normal alkyl hydroperoxides, 6 to 8 microns

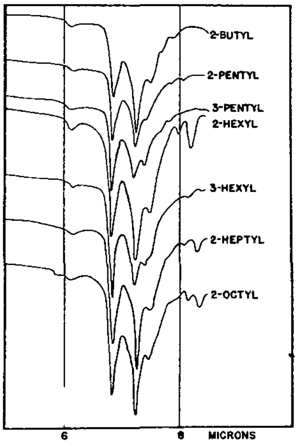

Figure 6. Comparison of infrared spectra of the secondary alkyl hydroperoxides, 6 to 8 microns

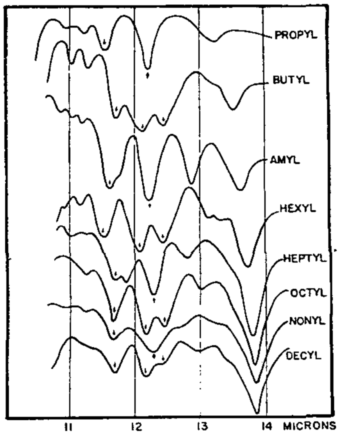

Figure 7. Comparison of infrared spectra of the normal alkyl hydroperoxides, 11 to 14 microns

Table V. Infrared Absorption by Alkyl Hydroperoxides in Region of 12.0 to 12.5 Microns

Hydroperoxide	Wave Length, μ
n-Propyl	12.20
n-Butyl	12.18, 12.45
n-Amyl	12.26
n-Hexyl	12.10, 12.46
n-Heptyl	12.26
n-Octyl	12.16, 12.46
n-Nonyl	12.26
n-Decyl	12.18, 12.42

shown in Figure 7. The hydroperoxides with an even number of carbons showed two maxima at about 12.1 and 12.4 microns, while those with an odd number of carbons showed a single maximum at about 12.3 microns. This is a very characteristic region for the normal hydroperoxide series and only in the case of *n*-octyl alcohol was there an interfering band with the alcohols and in this case it was very weak. This alternation in frequency must pertain to the hydroperoxide group since the liquid alcohols do not show this characteristic phenomena. No other reports of such an alternation in the infrared spectra of a homologous series taken in the liquid state is known to the authors. Sinclair (*8*) observed no systematic variations in the spectra of a series of even and odd chain length aliphatic acids. An extensive study of hydrocarbons by Sheppard and Simpson (*6*) has shown a similar regular alternation of infrared frequencies for even and odd carbon atom *n*-paraffinic hydrocarbons in the solid state, but these alternations were not distinguishable in the liquid state. These alternations in the solid *n*-paraffins (which are due to skeletal stretching modes) show the doublet for the odd carbon atom *n*-paraffin and the singlet for the even carbon atom homologs whereas the reverse of this is true in the above case of the *n*-alkyl hydroperoxides.

ACKNOWLEDGMENT

The authors wish to thank the California Research Corp. for a grant which made these studies possible.

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RECEIVED for review July 23, 1954. Accepted November 29, 1954.

Effect of Concentration and Sorption upon Migration of Cations in Paper Electrochromatography

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This investigation was designed to test the effect of concentration and sorption of cations upon their electrical migration through moist paper. Under the conditions employed in electrochromatography and with zones of ions such as lead and bismuth, that are readily sorbed by the paper, the rate of the electrical migration is least at the lowest concentration and increases with concentration, approaching a limit as the concentration of the ions in the migrating zones approaches the ionic concentration of the background electrolytic solution in the paper. In chromatographic tests based upon flow of the electrolytic solution through the paper, the migration of the zones of sorbed ions also increases with concentration. Consequently, the proportion of the ions sorbed by the paper must decrease with increasing concentration; conversely, the proportion of the ions remaining nonsorbed in the solution must increase with the increasing concentration. As the nonsorbed fraction of the ions should undergo faster electrical migration than the sorbed fraction, the electrical migration of all the ions should be proportional to the fraction not sorbed and should increase with concentration. For the precise description of migration rates, migration sequences, and separability, the concentration and the sorbability of the migrating ions should be specified. The effect of concentration upon the electrochromatographic separation of radium from its principal radioactive daughters and from barium has been determined.

IN PAPER electrochromatography, the separability of mixtures of various ions depends primarily upon the differential migration rates. These rates vary with many conditions such as the nature and concentration of the background electrolytic solution moistening the paper, the treatment and wetness of the paper, the temperature, and the magnitude of the electroosmotic effect (*3, 4, 6–8, 11, 14*).

Many ions, particularly those not sorbed by the paper, migrate at a rate independent of their concentration, provided the concentration is less than that of the background electrolytic solution (*7*). With zones of the mixture at concentrations higher than that of the electrolytic solution, the migration decreases with increasing concentration. At the leading boundaries of the zones, however, the ions diluted by diffusion and convection migrate rapidly, producing long diffuse leading regions (*7*).

Certain ions have now been found to migrate very slowly at low concentration, the rate of migration increasing with the concentration. With these ions at the lowest detectable concentration (as with carrier-free, radioactive isotopes), the rate of migration increases until the concentration approaches that of the background electrolytic solution. Only at concentrations significantly higher than that of the electrolytic solution do the migrating zones form diffuse, leading regions.

Separate chromatographic experiments with flow of solvent as the driving force have revealed a relationship between the sorbability of the ions by the paper and the rate of electrical migration. Ions such as phosphate migrate at a rapid rate with flow of the solvent and are, therefore, not sorbed by the paper. These ions exhibit a uniform rate of electrical migration at various levels of concentration below that of the background electrolytic solution (*7*). By contrast, ions such as bismuth and lead are transported at a slow rate by flow of solvent and are, therefore, sorbed by the paper (*9, 10*). The transport of these bismuth and lead ions by flow of solvent also increases with concentration; hence a smaller proportion of the ions must be sorbed at high concentration than at low concentration, a phenomenon frequently encountered and clearly revealed by the sorption isotherm (*9, 10*).

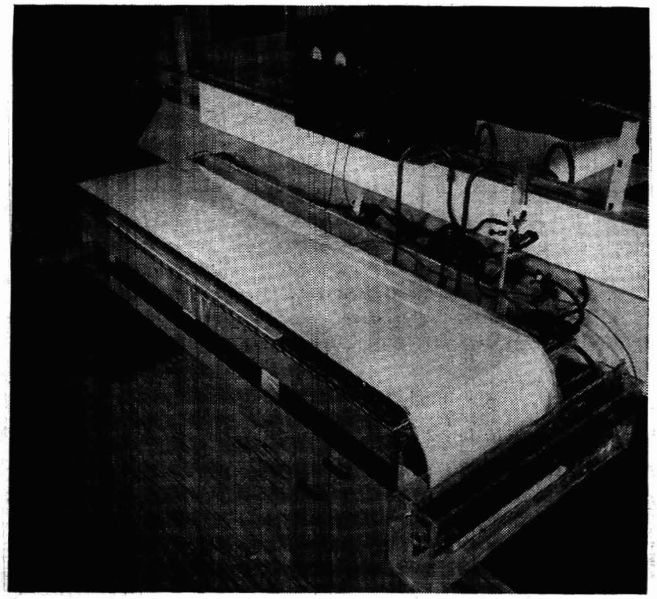

Figure 1. Apparatus for electrochromatography with paper 2 meters long

Lucite electrode vessels, graphite electrodes, lucite barrier between electrodes and paper, moistened paper in polyethylene wrapping supported on water-cooled, thermopane window

The variation of the sorption of ions with their concentration must now be considered in the specification of the electrical migration rates. This variation and its effect upon the rate of electrical migration must also be considered in the use of the electromigration sequences for the comparison and identification of substances even though the sequence is established in a single piece of paper (*8*).

APPARATUS

The electrical migration apparatus shown in Figure 1 is a modification of earlier models. The moistened paper wrapped in polyethylene sheeting (0.005 inch thick) was supported on a Thermopane window (nearly 1 or 2 meters long) cooled with running water.

The electrode vessels were made of plastic (Lucite). They were provided with electrodes of large, flat strips of graphite supported edgewise in the solution and separated from the paper by an upright sheet of plastic extending nearly to the bottom of the vessel. The direct current potential was obtained from an electronic rectifier (*7*) that provided about 800 volts.

MATERIALS

Carrier-free preparations of calcium-45, of strontium-90 with its daughter yttrium-90, and of barium-140 with its daughter lanthanum-140 were obtained from the Oak Ridge National Laboratory. Radioactive salts of lead-210 (Ra*D*), bismuth-210 (Ra*E*), and polonium-210 (Ra*F*) free of carrier and free of the parent radium were obtained from radon. The solutions with decay products of radon provided about 50,000 counts per minute per 10 μl. of solution (about 0.1 microcurie). These carrier-free elements were also obtained by the use of aged solutions of radium salts (usually 5 to 50 γ of radium per 100 μl.). In these solutions, only those decay products with a long half life (indicated by bold face in the following series), were detectable by autographic methods:

$$\mathbf{Ra^{226}} \rightarrow \mathrm{Rn}^{222} \rightarrow \mathrm{Po}^{218} \begin{matrix} \nearrow \mathrm{At}^{218} \searrow \\ \searrow \mathrm{Pb}^{214} \nearrow \end{matrix} \mathrm{Bi}^{214}$$

$$\begin{matrix} \nearrow \mathrm{Po}^{214} \searrow \\ \searrow \mathrm{Tl}^{210} \nearrow \end{matrix} \mathbf{Pb^{210}} \rightarrow \mathbf{Bi^{210}} \begin{matrix} \nearrow \mathbf{Po^{210}} \searrow \\ \searrow \mathrm{Tl}^{206} \nearrow \end{matrix} \mathrm{Pb}^{206} \text{ (inactive)}$$

The paper employed in these experiments was Eaton-Dikeman, grade 301, 0.03 inch thick. This wood-pulp paper was employed without treatment and also after thorough extraction by downward percolation with (1*M*) nitric acid, (*1M*) acetic acid, and water. The unwashed paper contained about 0.12% ash which was some 46% calcium. The washed and dried paper contained about 0.01% ash which was about 0.9% calcium. The paper strips were several centimeters longer than 1 or 2 meters and were arranged so that there were either 1 or 2 meters of paper between the solutions in the electrode vessels.

PROCEDURES

The relative electrical migration rates of the ions at various concentrations were determined in wide strips of paper. No attempt was made to determine the absolute rates or the effect of electroosmosis upon the migration (*14*).

Before use, the paper was moistened with the electrolytic solution, usually 0.1*M* lactic acid. It was blotted lightly and wrapped in the polyethylene sheet, and the ends were placed in the electrolytic solution (about 8 liters in each electrode vessel). With the polyethylene wrapping opened momentarily,

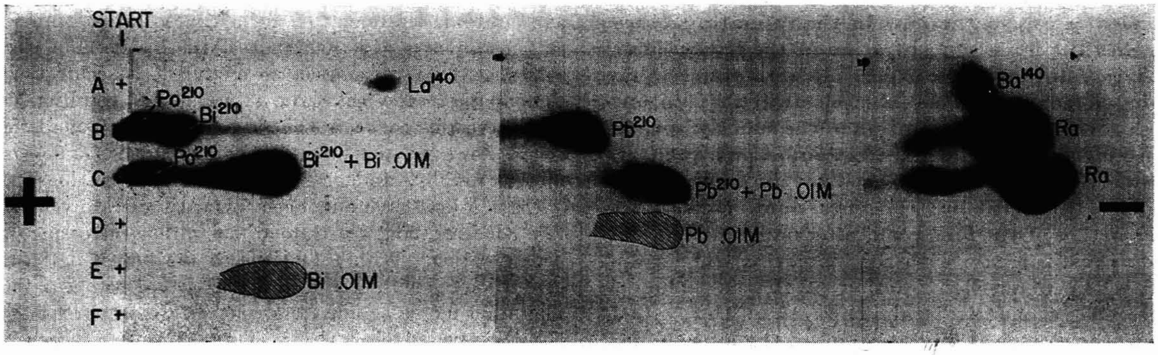

Figure 2. Autograph of electrochromatogram showing effect of concentration upon electrical migration of various ions

A. Barium and lanthanum
B. Radium and long-lived decay products
C. Radium with 0.01*M* lead and bismuth
D, E. 0.01*M* lead and bismuth located with hydrogen sulfide
F. Blank

Potential. 700 volts per 200 cm.
Migration time. 23.5 hours
Electrolyte. 0.1*M* lactic acid

A to *F*. 25 cm.
Unwashed filter paper
Ra contained decay products

the solutions to be examined (about 50 μl. each) were added at marked starting points, as indicated by the figures, and the migration was carried out for some 20 to 24 hours with 3.5 or 7 volts per cm. After the migration, the zones containing nonradioactive ions were located by spraying the paper with solutions of sodium sulfide or hydrogen sulfide for lead and bismuth and with sodium rhodizonate for barium. The paper was then dried, and the zones of radioactive ions were located by autographic methods using several juxtaposed sheets of Kodak No-screen x-ray film 14 × 17 inches (exposure about 8 to 16 hours).

RESULTS

The relative migration of barium and its daughter lanthanum is shown in Figure 2, *A*. The migration of radium-226 and of its decay products lead-210, bismuth-210, and polonium-210 are shown in Figure 2, *B* (trailing portions of radium zones not identified). Addition of lead and bismuth ions (each 0.01*M*) to the radium solution before the migration increased the rate of migration of the tracers as shown in Figure 2, *C*. Separate zones of nonradioactive lead and bismuth ions (each 0.01*M*) migrated at the same rate as the zones of tracer plus the 0.01*M* solutions (Figure 2, *D*, *E*). The radioactive lead zones exhibited distinct trailing regions as indicated by the figure. Similar trailing regions were observed when the zones formed with 0.01*M* lead solutions were located with sulfide (Figure 2, *D*, *E*).

Separations of lead, bismuth, and polonium similar to those illustrated in Figure 2 were also obtained with the radioactive daughters of radon. The migration of these lead and bismuth zones was likewise accelerated by the addition of 0.01*M* lead and bismuth solutions to the mixture of tracers before the migration.

The effect of concentration upon the migration of bismuth ions is illustrated in Figure 3. Bismuth from radium (or from radon) migrated extremely slowly (Figure 3, *D*). The rate of migration increased progressively with the addition of 0.0001*M*, 0.001*M*, and 0.01*M* bismuth solutions. As in the previous illustration, the tracer plus the inactive carrier (Figure 3, *A* to *C*) migrated at the same rate as the carrier alone (Figure 3, *E* to *F*). This rate was determined primarily by the concentration of the carrier. In all these comparative migrations, the bismuth zones became elongated yielding large trailing regions. As might be expected, the addition of the bismuth to the mixture of the tracers had no effect upon the trailing of the zones or upon the rate of migration of the lead.

The extent to which the migrating radium remained in a single zone was determined by estimation of the γ-ray activity. To this end, aliquot portions of a dilute radium solution were sealed in glass tubes. Similar aliquot portions of the solution were submitted to electrical migration, and after the migration, the

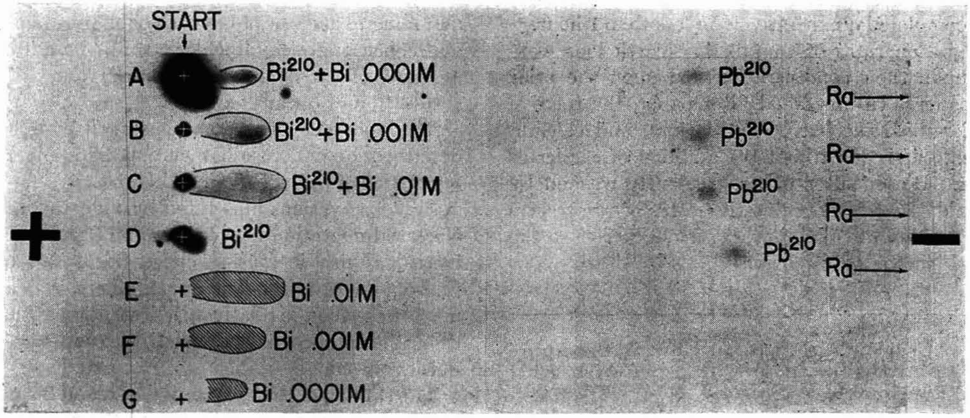

Figure 3. Autograph of electrochromatogram showing effect of concentration upon electrical migration of bismuth ions

A to *C*. Bismuth tracer plus bismuth carrier, polonium remains at start
D. Bismuth and polonium tracers remain at start
E to *G*. Bismuth located with hydrogen sulfide

Potential. 700 volts per 100 cm.
Migration time. 24 hours
Electrolyte. 0.1*M* lactic acid (in vessels)
A to *G*. 30 cm.

Unwashed filter paper
A,*B*,*C*,*D*. Radium decay products
A,*D*. Po^{210} trailed Bi^{210}
Ra off paper

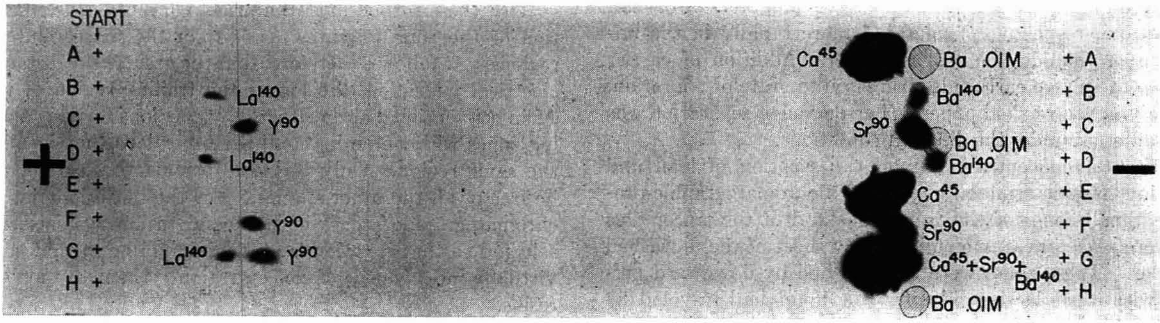

Figure 4. Autograph of electrochromatogram showing effect of concentration upon electrical migration of barium, strontium, and calcium ions in unwashed filter paper

Barium in hatched regions located with rhodizonate. Yttrium and lanthanum daughters of strontium and barium

Potential. 700 volts per 200 cm.
Migration time. 24 hours
Electrolyte. 0.05*M* lactic acid
A to *H*. 35 cm.
Unwashed filter paper
A. Ca^{45} plus Ba

B. Ba^{140}
C. Sr^{90} plus Ba
D. Ba^{140}
E. Ca^{45}
F. Sr^{90}

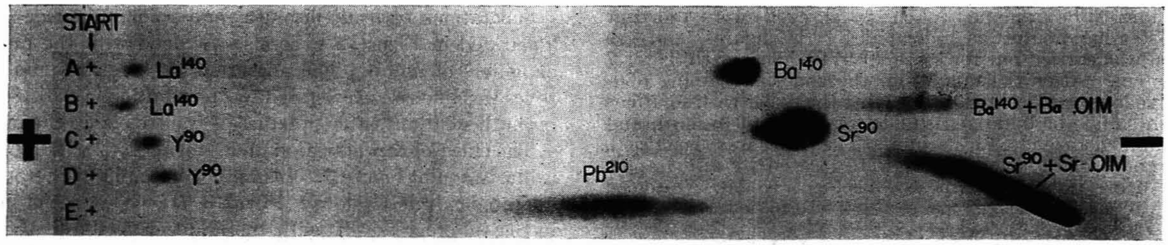

Figure 5. Autograph of electrochromatogram showing effect of concentration upon electrical migration of barium and strontium ions in washed filter paper

Potential. 700 volts per 200 cm.
Migration time. 21 hours
Electrolyte. 0.05*M* lactic acid
A to *E*. 20 cm.
Washed filter paper

portions of the paper containing the radium were cut out and were also sealed in glass tubes. After several weeks, the γ-ray activity of all the samples was determined with a pressurized ionization chamber. In 12 determinations, 97 to 100% of the radium was always obtained in the principal radium zone after migration.

Small scale separations of radium from its daughters were performed rapidly in hardened filter paper moistened with 0.1*M* lactic acid. With as little as 1 μl. of solution containing only 0.05 γ of radium, the separation of radium, lead, bismuth, and polonium was completed quantitatively in less than 1 hour.

The relative migration rates of the alkaline earth ions were found to depend upon their concentration and upon the prior treatment of the paper. In unwashed filter paper, the relative migration rates of radioactive barium, strontium, and calcium were nearly the same with or without the addition of carrier as shown in Figure 4. But in filter paper previously washed by percolation with acid, the relative migration rates of the tracers were increased by the addition of nonradioactive carrier to the tracer as shown in Figure 5.

Table I. Effect of Concentration upon Chromatographic *R* Values of Lead and Bismuth Ions in Paper with 0.1*M* Lactic Acid as Solvent

Ion	Concentration, *M*	*R*	1−*R*
Pb	Tracer	0.49	0.51
	0.0001	0.53	0.47
	0.001	0.69	0.31
	0.10	0.80	0.20
Bi	Tracer	0.21	0.79
	0.0001	0.36	0.64
	0.001	0.57	0.43
	0.10	0.63	0.37

Lanthanum and yttrium, the radioactive daughters of barium and strontium, respectively, migrated more rapidly in the unwashed paper than in the washed paper. Addition of carrier (0.01*M*) to these rare earths also increased the rate of migration, particularly in the washed paper. The degree of separation was not very different under the different conditions.

The effect of concentration upon the sorption of lead and bismuth ions was determined by standard chromatographic procedures. Small zones of the tracer ions and of the tracer plus carrier were placed near one end of a dry sheet of the unwashed filter paper. These zones were then washed by downward percolation with 0.1*M* lactic acid until the liquid had traveled 84 cm. The excess paper was cut off at the liquid front. The moist portion was dried; the zones of the ions were located by autoradiography; and the distance of migration relative to the migration of the solvent (the *R* value) was determined (Table I).

DISCUSSION

From Table I, the *R* values for bismuth and lead ions increase with concentration. Because the *R* value is directly proportional to the fraction of the ions that remains nonsorbed in the solution (*10*), this increase of *R* with the concentration of lead and bismuth ions shows that an increasing proportion of the ions remains nonsorbed as the concentration increases. And as indicated by the values of 1-*R* in Table I, a decreasing proportion of the ions is sorbed as the concentration increases.

The decrease in the relative sorbability of the ions with increasing concentration is in agreement with analogous chromatographic observations by Frierson and Jones (*2*). These investigators found that, with solvents composed of butyl alcohol and mineral acids or of butyl alcohol, pyridine, acetic acid, water, and concentrated hydrochloric acid, the addition of unspecified amounts of carrier increased the *R* values of radium *D* and *E* (lead-210 and bismuth-210).

The above effect was not observed by Dickey (*1*), who has described an independent chromatographic procedure for the separation of radium *D*, *E*, and *F*.

The observations illustrated by Figures 2 and 3 show that the electrical migration rate of lead and bismuth ions in moist paper increases with increasing concentration.

From the results in Table I, this increase in electrical migration may be ascribed to relatively less sorption at the higher concentrations, the nonsorbed ions being free to undergo electrical migration in the solution.

The trailing of the radioactive lead and bismuth zones as shown in Figure 2 may be attributed to the relatively strong sorption at low concentration and also to the formation of decay products as the radium migrates through the paper.

With nonradioactive lead and bismuth ions, however, long trailing zones could also be detected with hydrogen sulfide. In this case, the trailing must be due, primarily, to the relatively greater sorbability (and possibily also to the lower rate of elution) of the lead and bismuth ions at the low concentrations.

With these sorbed ions, the principal, more concentrated portion of the zone migrates faster than the more dilute trailing regions. It follows, therefore, that the greater the distance of the electrical migration, the longer the trailing region of the zone. As in conventional chromatography, the ions forming these trailing zones will contaminate all the other ions that form zones in the same region. Only the ions forming the leading zone will be free of all the other species. For the most complete electrochromatographic separations, the migration systems should be selected so that sorption is at a minimum or so that sorption is virtually independent of concentration as in many ion exchange resins.

The small differences among the migration rates of the alkaline earth ions illustrated by Figure 4 may be attributed to the calcium ions in the unwashed paper. These calcium ions displace the alkaline earth tracers which then migrate at a more rapid rate analogous to the migration of the carrier-containing solutions shown in Figure 5.

The observations summarized by Figures 2 and 3 represent practical procedures for the separation of radium from its longerlived daughters. In principle, this separation is similar to the separation of radium and its daughters by electrical migration in a gel described by Veil in 1934 (*13*).

As shown by numerous experiments like those illustrated by Figures 2 and 5, the separations of radium from barium and of barium from strontium were usually incomplete. These separations were most effective at low concentration and in washed filter paper wherein sorption plays an effective role. By contrast with chemical methods of separation, barium ions do not serve as a carrier for radium ions or for strontium ions during the differential electrical migration.

The ion exchange column separation of radium and barium has recently been improved by the use of suitable complex-forming anions such as citric acid (*5, 12*). By analogy, the use of similar complex-forming substances in the electrolytic solution may facilitate the separation of barium and radium by electrical migration.

ACKNOWLEDGMENT

The authors are indebted to T. W. Speckman, who determined the γ-ray activity of the radium samples.

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Received for review August 30, 1954. Accepted December 8, 1954.

Polarographic Determination of *S*-(1,2-Dicarbethoxyethyl)-*O*,*O*-Dimethyl Dithiophosphate (Malathion)

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A polarographic method for the assay of technical grade malathion is described. Unreacted diethyl fumarate is first determined in 0.3*N* hydrochloric acid–30% ethyl alcohol solution, in which malathion is polarographically inactive. On treatment with alkali, both unreacted ester and malathion are quantitatively hydrolyzed to fumaric acid which is also determined polarographically after acidification of the solution. The contribution of the unreacted ester to the fumaric acid wave may be calculated and the corrected fumaric acid wave computed to its equivalent malathion. Evidence of film formation at the dropping mercury electrode by malathion, with resulting displacement of the waves of maleic and fumaric acids, their ethyl esters, oxygen, and hydrogen peroxide towards more negative potentials, is discussed. The half-wave potential of thallous ion, however, remains unchanged in the presence of malathion.

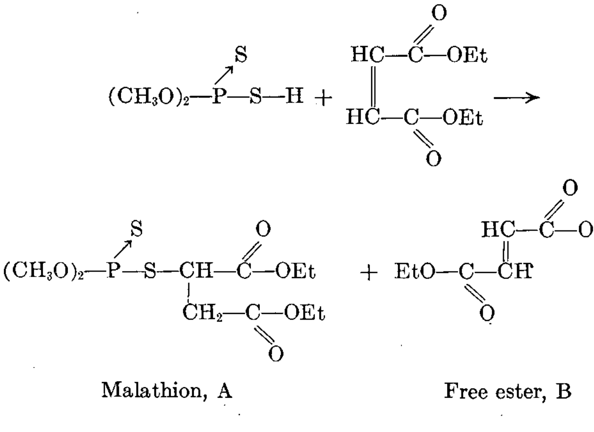
THE discovery that *S*-(1,2-dicarbethoxyethyl)-*O*,*O*-dimethyl dithiophosphate, commercially known as malathion, was a potentially valuable insecticide, and the decision to investigate its manufacture and application, necessitated the development of methods of analysis applicable to the technical product and to its determination as residue on plant materials.

For residue analysis, Averell and coworkers (*1*) have worked out a colorimetric method which is based upon the alkaline decomposition of the insecticide and reaction of copper(II) with the *O*,*O*-dimethyl ester of phosphorodithioic acid to form an intense yellow-colored complex in carbon tetrachloride.

The colorimetric method has also been extended to the analysis of formulations and the relatively pure product. However, for the analysis of crude samples, such as were encountered in process development studies, the colorimetric method was not satisfactory.

In this paper, a polarographic method for the determination of malathion is described. One of its greatest advantages has been its successful application, for more than two years, to a wide variety of samples. An advantage, in so far as technique is concerned, is the fact that the reaction conditions do not have to be as rigorously controlled as in the case of the colorimetric method. This is due to the fact that the polarographic method is based upon the determination of the stable breakdown product—i.e., fumaric acid—and not on the determination of the *O*,*O*-dimethyl ester of phosphorodithioic acid, which is unstable and undergoes partial decomposition in the alkaline medium used to break down the insecticide.

Malathion is prepared (*2*) by the reaction of the *O*,*O*-dimethyl ester of phosphorodithioic acid with diethyl maleate (Reaction 1).



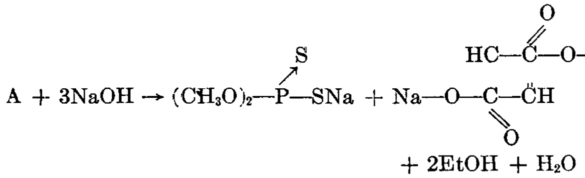
$$(CH_3O)_2\overset{S}{\overset{\nearrow}{P}}—S—H + \begin{matrix} HC—\overset{O}{\overset{/\!/}{C}}—OEt \\ \| \\ HC—\underset{\underset{O}{\diagdown\!\diagdown}}{C}—OEt \end{matrix} \longrightarrow$$

$$(CH_3O)_2\overset{S}{\overset{\nearrow}{P}}—S—\begin{matrix} CH—\overset{O}{\overset{/\!/}{C}}—OEt \\ | \\ CH_2—\underset{\underset{O}{\diagdown\!\diagdown}}{C}—OEt \end{matrix} + EtO—\underset{\underset{O}{\diagdown\!\diagdown}}{C}—\begin{matrix} HC—\overset{O}{\overset{/\!/}{C}}—OEt \\ \| \\ CH \end{matrix} \quad (1)$$

Malathion, A

Free ester, B

Diethyl fumarate, resulting from the isomerization of the maleate, may be expected as an impurity in the technical product. This unreacted ester, henceforth referred to as "free ester," may, however, be readily distinguished from malathion because it is polarographically active in acid solution—a medium in which malathion is stable and gives no polarographic waves. Diethyl fumarate is hydrolyzed to disodium fumarate by sodium hydroxide and, under the same conditions, it was found that malathion was rapidly and quantitatively broken down to the sodium salt of the *O,O*-dimethyl ester of phosphorodithioic acid and disodium fumarate by a base-catalyzed elimination reaction (Reaction 2).



$$\mathrm{A + 3NaOH \rightarrow (CH_3O)_2\overset{\overset{S}{\nearrow}}{P}{-}SNa + Na{-}O{-}\underset{\underset{O}{\searrow}}{C}{-}\overset{\overset{HC-\overset{\overset{O}{\swarrow}}{C}-O-Na}{\|}}{CH} + 2EtOH + H_2O} \qquad (2)$$

Utilizing the above chemical properties, the polarographic assay of technical malathion consists of: examining the sample in acid solution (0.3*N* hydrochloric acid, 30% ethyl alcohol) to determine the free ester, and hydrolyzing both the free ester and malathion with alkali, acidifying the solution, and determining the total fumaric acid. The determination of the free ester makes it possible to subtract its contribution to the fumaric acid wave. The corrected fumaric acid wave thus represents the acid resulting from the hydrolysis of the insecticide and may then be calculated to its equivalent malathion.

APPARATUS

A Leeds and Northrup Electrochemograph, Type E, was used throughout this work.

Analyses were carried out in a Heyrovský cell. Connection to the external saturated calomel electrode (S.C.E.) was made via an agar–potassium chloride bridge.

Oxygen was removed from the sample solution with Airco prepurified nitrogen which was saturated with the sample solution by passing it through a reservoir containing a portion of the solution in the Heyrovský cell.

A Serfass conductivity bridge, Model RC-M 15, was used to measure the cell resistance of all solutions studied. In every case, the *IR* drop was negligible.

Table I. Calibration Data for Hydrolyzed Malathion

Malathion Hydrolyzed, Gram[a]	μa.[b]	Mg. Malathion per 100 Ml. of Solution per μa.
0.01212	1.25	9.74
0.02116	2.17	9.77
0.02424	2.48	9.79
0.03096	3.16	9.85
0.03526	3.59	9.81

[a] Final volume of solution is 100 ml. as per procedure outlined.
[b] Corrected for residual.

REAGENTS

Ethyl alcohol, 95%.
Hydrochloric acid, 3*N*.
Sodium hydroxide, 0.1*N*.
Diethyl fumarate, Eastman Kodak (white label).

Malathion, highly purified material obtainable from American Cyanamid Co., Stamford, Conn. Purification procedure analogous to that used for parathion (*7*). Boiling point = 156–7° C. at 0.7 mm. of mercury with decomposition; refractive index n_D^{25} = 1.4985; specific gravity = 1.2284 at 26.5/26.5° C. Complete physical data will be published at a later date.

PROCEDURE

Preparation of Typical Calibration Curve for Malathion. The malathion used in the preparation of the calibration curve was a highly purified sample prepared in these laboratories. It was subsequently shown that Eastman Kodak diethyl fumarate (white label grade) could be used for calibration purposes.

Table II. Calibration Data for Free Ester in Malathion

Standard	Diethyl Fumarate, Mg.[a]	Ester, %	μa.[b]	Mg. Ester per 50 Ml. of Solution per μa.
1	0.716	1.95	0.277	2.59
2	2.56	6.64	0.964	2.65
3	5.11	12.5	1.966	2.60

[a] Mg. of diethyl fumarate per 0.0359 gram of malathion per 50 ml. of 30% ethyl alcohol, 0.3*N* hydrochloric acid.
[b] Corrected for residual.

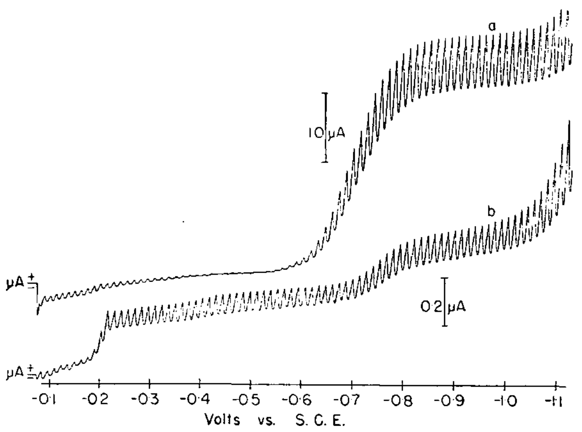


Figure 1. Typical polarograms of malathion sample before and after hydrolysis

a. Malathion sample hydrolyzed by alkali. Solution: 0.3*N* hydrochloric acid, 25% ethyl alcohol, oxygen removed
b. Malathion sample containing 1.1% diethyl fumarate. Solution: 0.3*N* hydrochloric acid, 30% ethyl alcohol, oxygen removed

Samples of approximately 0.1 to 0.35 gram (weighed to nearest milligram) were weighed, dissolved, and diluted to 250 ml. with 95% ethyl alcohol This gave solutions of malathion ranging from 0.001 to 0.004*M*. A 25.0-ml. aliquot of a given standard solution was put in a 100-ml. volumetric flask. To this were added 25 ml. of 0.1*N* sodium hydroxide. After a hydrolysis time of 3 minutes, the contents were acidified with 10 ml. of 3*N* hydrochloric acid. The solution was then diluted to 100 ml. with distilled water at 25° C. A portion of this solution was then placed in a Heyrovský cell, freed of oxygen, and the dropping mercury electrode polarized from −0.1 volt *vs.* S.C.E. to hydrogen ion discharge. A blank was also run to provide the residual current correction. Table I shows data obtained in a typical calibration.

Preparation of Typical Calibration Curve for Unreacted Diethyl Fumarate. A 1.91-gram sample of highly purified malathion (free of diethyl fumarate) was weighed, transferred to a 200-ml. volumetric flask, and diluted to the mark with 95% ethyl alcohol (Solution A). Into a 100-ml. volumetric flask, 0.1705 gram of diethyl fumarate was transferred and diluted to the mark with 95% ethyl alcohol (solution B). In each of four 100-ml. volumetric flasks, 25.0 ml. of Solution A were placed. To these solutions, 0.0, 2.8, 10.0, and 20.0 ml. of Solution B were added and the solutions diluted to 100 ml. with 95% ethyl alcohol. These solutions were equivalent to approximately 0.25 gram of samples of insecticide containing 0, 2, 6, and 12% free ester.

A 15-ml. aliquot of each of these solutions was pipetted into a 50-ml. volumetric flask, 5.0 ml. of 3*N* hydrochloric acid added, and the solution diluted to the mark at 25° C. A portion of the solution was placed in a Heyrovský cell, freed from oxygen, and the dropping mercury electrode polarized from −0.1 volt *vs.* S.C.E. to hydrogen ion discharge. Table II shows data obtained in a typical calibration.

Analysis of Technical Grade Malathion. Weigh out 0.2 to 0.25 gram (to the nearest milligram) of sample into a 100-ml. volumetric flask and dilute to the mark with 95% ethyl alcohol. Pipet a 15-ml. aliquot into a 50-ml. volumetric flask, add 5 ml. of 3*N* hydrochloric acid, and dilute to 50 ml. with water. Deaerate as indicated earlier and polarize from −0.1 volt *vs.* S.C.E. to hydrogen ion discharge. The percentage of diethyl fumarate may be calculated with the aid of the calibration curve.

Weigh out approximately 0.30 gram (to the nearest milligram) of sample into a 250-ml. volumetric flask and dilute to the mark with 95% ethyl alcohol. Pipet a 25-ml. aliquot of this solution into a 100-ml. volumetric flask and add 25 ml. of $0.1N$ sodium hydroxide. After 3 minutes—longer time is harmless—acidify the solution with 10 ml. of $3N$ hydrochloric acid and dilute to the mark with water. Deaerate a portion of the solution and polarize the dropping mercury electrode from −0.1 volt *vs.* S.C.E. to hydrogen ion discharge. After subtracting the contribution of the hydrolyzed free ester to the fumaric acid wave, the malathion content may be computed with the aid of calibration data.

All waves are corrected for residual currents and typical results obtained on technical grade malathion are tabulated in Table III.

In Figure 1, curve *b*, is shown a typical polarogram obtained in the determination of the free ester in a technical grade sample. The material responsible for the small wave appearing at approximately −0.2 volt *vs.* S.C.E. has never been identified.

Figure 1, curve *a*, shows the polarogram obtained after acidification of a hydrolyzed solution of the same sample.

DISCUSSION

Early in the polarographic work on this problem, it was noticed that malathion had the capacity of displacing the polarographic waves of many substances towards more negative potentials by as much as 0.1 to 0.3 volt. For example, the waves of maleic acid, fumaric acid, diethyl maleate, and diethyl fumarate (at concentrations of approximately $0.00015M$) were all shifted towards more negative potentials in a medium of 30% ethyl alcohol, $0.3N$ hydrochloric acid, containing $0.00227M$ malathion (Figure 2). In the presence of malathion, and under the same conditions, the wave representing the electroreduction of oxygen to hydrogen peroxide is shifted to more negative potentials by 0.3 volt; the second wave, representing the reduction of hydrogen peroxide, is also shifted toward more negative potentials by approximately 0.2 volt and appears to be more reversible. Heyrovský (*5*) also observed that when hydrogen peroxide is reduced in the presence of camphor—a strongly capillary-active material—the wave is shifted to more negative potentials and appears to be more reversible.

Table III. Typical Results Obtained by Polarographic Analysis Malathion (Technical Grade)[a]

Sample	Malathion, %	Diethyl Fumarate, %
CS 1	93.5, 90.8	2.46, 2.42
CS 4	86.6, 85.9, 87.1	3.70, 3.66
CS 5	87.5, 88.6, 88.5	2.45, 2.38
CS 9R	91.9, 91.1	2.18, 2.26
CS 13R	89.4, 88.7	3.37, 3.47
CS 16R	89.6, 89.1	2.70, 2.76
CE-18-493	87.8, 88.2	4.53, 4.57

[a] Each analysis represents an analysis of separate sample and not an analysis of separate aliquots of a single master solution of the sample in question.

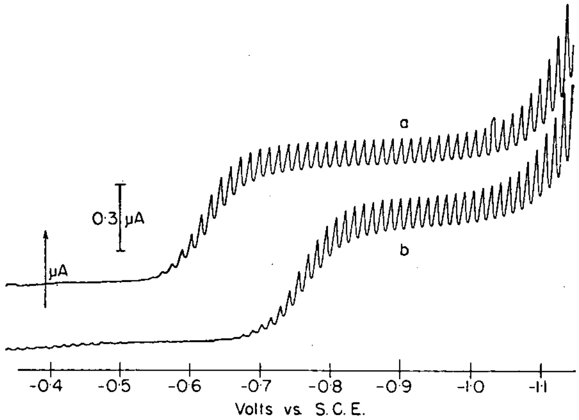


Figure 2. Displacement of diethyl fumarate wave in presence of malathion

a. Oxygen-free solution. $0.00015M$ diethyl fumarate, $0.3N$ hydrochloric acid, 30% ethyl alcohol
b. Oxygen-free solution. $0.00015M$ diethyl fumarate, $0.3N$ hydrochloric acid, 30% ethyl alcohol, $0.00227M$ malathion

Table IV. Effect of Malathion on the Electrocapillary Curve of a Solution of 0.3N Hydrochloric Acid, 30% Ethyl Alcohol

	Seconds per 10 Drops		
E_{applied} *vs.* S.C.E., Volt	30% EtOH, 0.3 NHCl	30% EtOH, $0.3N$ HCl, $0.00015M$ diethyl fumarate	30% EtOH, $0.3N$ HCl, $0.00015M$ diethyl fumarate, $0.00227M$ malathion
−0.1	40.4	40.4	39.8
−0.2	41.7	41.5	40.2
−0.3	41.9	41.9	40.2
−0.4	42.0	42.0	40.2
−0.5	41.5	41.8	40.0
−0.6	41.2	41.3	40.0
−0.7	40.7	40.9	40.0
−0.8	40.4	40.3	39.5
−0.9	39.8	39.7	39.4
−1.0	39.4	39.0	39.2
−1.1	38.6	38.1	38.9

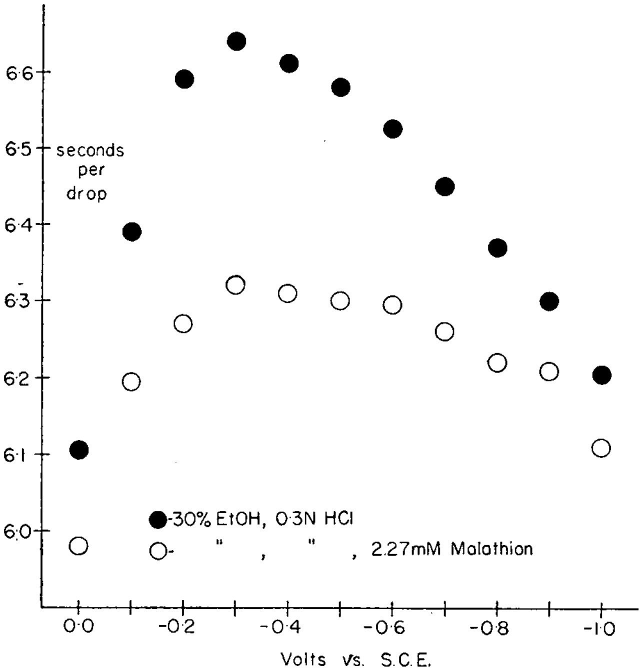


Figure 3. Electrocapillary curves showing suppressive effect of malathion

Heyrovský and coworkers (*6*) obtained oscillographic potential-time curves by polarizing the dropping mercury electrode or a streaming mercury electrode with an alternating current. The resulting patterns showed marked time lags when certain electrolytically inactive substances, such as pyridine in alkaline solutions, are present. These same substances also caused a displacement of polarographic waves towards more negative potentials, and it was concluded by Heyrovský and coworkers that the phenomenon was due to the formation of a film of nonelectrolyte (pyridine) adsorbed at the electrode surface The electrode processes of several cations and nitrobenzene were found to be hindered by the adsorbed film and proceeded freely only with a breakdown of the film which occurred at more negative potentials. An exception to these results was found, by the same workers, in the case of the behavior of thallous ion. It was found that the reduction process of the thallous ion was unaffected by the presence of the pyridine film. Identical results were obtained by the present author in the electroreduction of the thallous ion in the presence of malathion. The half-wave potential of the thallous ion wave was found to be the same in the presence and absence of malathion.

Capillary activity of malathion is further indicated by its suppressive effect on the electrocapillary curve as indicated by the data in Table IV and by Figure 3.

Table V. Effect of Ethyl Alcohol Content on Half-Wave Potentials of Maleic and Fumaric Acids and Their Ethyl Esters[a]

95% Ethyl Alcohol, Volume %	Half-Wave Potential, Volt *vs.* S.C.E.			
	Maleic acid	Fumaric acid	Diethyl maleate[b]	Diethyl fumarate
0	−0.598	−0.605	...	...
10	−0.594	−0.678	−0.767	−0.621
20	−0.611	−0.734	−0.833	−0.646
30	−0.633	−0.770	−0.884	−0.674
40	−0.654	−0.808	−0.930	−0.716
50	−0.667	−0.834	...	...
60	−0.676	−0.855	...	...
70	−0.672	−0.868	...	...

[a] Solutions were $0.5mM$ in electroactive component, $0.1N$ hydrochloric acid, temperature was 25° ± 0.1° C., and solutions were free of oxygen. Nitrogen was saturated with portion of sample before it was passed through polarographic cell.

[b] 0.005% sodium methyl red present to suppress maximum.

A number of compounds were examined to ascertain their effect on the diethyl fumarate and malathion determinations. No interferences were found.

In the presence of malathion, diethyl maleate is reduced at a more negative potential than diethyl fumarate. If present, it could readily be distinguished from, and determined along with, the diethyl fumarate. Only in very special experiments have both esters been found. The presence of maleic and fumaric acids is not expected in view of the conditions under which the preparation of malathion is carried out. Unreacted *O,O*-dimethyl ester of phosphorodithioic acid and the corresponding disulfide are without effect on either the free ester and malathion determinations. It was thought that the wave appearing at −0.2 volt *vs.* S.C.E. in unhydrolyzed solutions (Figure 1, curve *b*) was due to the disulfide, but the half-wave potentials were not the same.

While the effect of malathion on the half-wave potentials of maleic and fumaric acids and their ethyl esters was studied, it was observed that a separation of the maleic and fumaric acid waves could be effected in acid solutions simply by increasing the alcohol content to approximately 40 to 50%. Table V shows the half-wave potentials obtained for $0.0005M$ concentrations of these acids and their ethyl esters for different concentrations of ethanol in $0.1N$ hydrochloric acid (*IR* drop negligible in all cases). While an explanation for the separation of the acid waves is beyond the scope of the present paper, the data are offered, inasmuch as they may prove useful where it is not feasible to work in the ammonia–ammonium chloride buffer (pH 8.2) system which is usually employed for the differentiation of the maleic and fumaric acids (*3, 4*).

ACKNOWLEDGMENT

The helpful discussions with Emil F. Williams of these laboratories are gratefully acknowledged.

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RECEIVED for review June 11, 1954. Accepted December 10, 1954. Presented before the Division of Analytical Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, September 1954.

Identification of Curing Agents in Rubber Products

Ultraviolet Absorptiometric Analysis of Selective Solvent Extracts

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A method has been devised for identification of organic compounds that accelerate the vulcanization of rubber products. Identification of these active trace materials aids product improvement and quality control. Identification is made through ultraviolet spectrophotometric absorbance curves over the 220 to 380 mμ region on the aqueous alkali or acid extracts, or on liquid-liquid ethyl ether extracts of the aqueous solutions. Thiazole, thiuram, thiocarbamate, amine, and guanidine classes of commercial accelerators are regularly identified in 2 grams of uncured or cured rubber products with less than 4 hours' elapsed time. Interference of common softeners and antioxidants is usually negligible. The method is more rapid than chromatographic procedures and more sensitive and specific than spot test methods. Quantitative results may be obtained if calibration work is undertaken.

PREVIOUS published methods used for accelerator identification in compounded rubber products have fallen into four classes: chemical spot tests or color reactions, precipitation and melting point tests, chromatography of colored accelerator products, and spectrophotometric identification of chromatographed fractions.

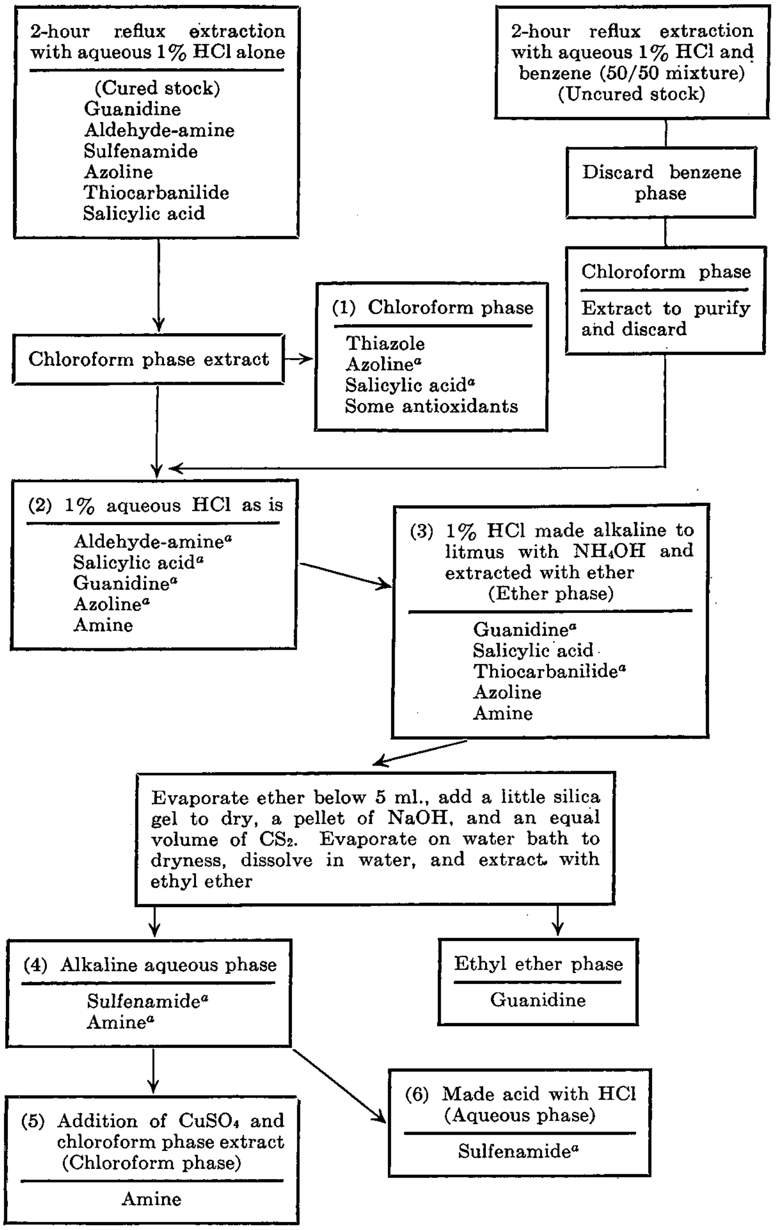
Spot tests or colorimetric methods have been reported for 2-mercaptobenzothiazole and other accelerators (*1, 3, 13, 18–20*). Most of these methods were applied to the acetone extracts of large amounts of the rubber compound, and the formula seldom was as complex in softener and antioxidant content as today's commercial products.

The precipitation of guanidine picrates and their identification by melting point have been well developed (*8, 21*). Precipitation of thiazoles as the cadmium salts has also been reported (*15*). It has been established in this laboratory that the ultraviolet spectrophotometric method is at least 10 times more sensitive than the cobalt oleate spot test for 2-mercaptobenzothiazole. It has also proved more sensitive and reliable for guanidine identification in today's complex stocks than the picrate melting point method (*12*).

Ultraviolet spectrographic techniques have been used by Dufraisse and Houpillart (*5*) in following the progress of 2-mercaptobenzothiazole and 2,2'-benzothiazyl disulfide in the curing of simple rubber products, and Jarrijon (*9*) has used the same technique in following the transformation of tetramethylthiuram disulfide to zinc dimethyldithiocarbamate during vulcanization.

Extensive work has been reported by Koch (*10*) on spectral absorption and structure of organic sulfur compounds. The ultraviolet absorbance curves of a number of sulfur-bearing materials used as accelerators in the rubber industry are given.

Table I. Outline A. Procedure for Accelerator Identification with Acid Extract



[a] Major or most reliable test. Other tests are more or less confirmatory and are not always needed for identification.

Ultraviolet spectrophotometry has been used for analysis of accelerator masterbatches by Kress (*11*), while Fink, Field, and coworkers (*6*) have combined chromatography and ultraviolet spectrophotometry in the determination of 2-mercaptobenzothiazole in a cured compounded stock.

Hilton and Newell (*7*) have determined the amount of thiurams and dithiocarbamates in a rubber compound by the ultraviolet spectrophotometric analysis of products formed from the decomposition of thiocarbamates to carbon disulfide by acidulated alcohol.

Infrared absorption spectroscopy has been successfully used by Mann (*14*), but all work had to be preceded by time-consuming chromatographic separations. Mann was of the opinion that ultraviolet spectroscopy and polarography were more promising tools than infrared in this respect. Preliminary work using polarographic techniques has been reported for a number of accelerators (*17*).

In most of the reported techniques for the determination of accelerators involving chromatography, much time is consumed in the initial separation (*2*, *16*), while other methods are not sensitive enough to detect small quantities (*6*). Some methods make no allowance for the softeners and antioxidants now commonly incorporated in a rubber compound, which in the case of spot tests could introduce serious errors. Many methods now used require a large quantity of sample which is not always available.

OUTLINE OF METHOD

In the method outlined (Tables I and II) the accelerator is directly and rapidly extracted from the stock, with little interference from other compounding ingredients. The various classes are identified by matching the ultraviolet spectral curve of the unknown with that of a similar selective solvent preparation of known compounds.

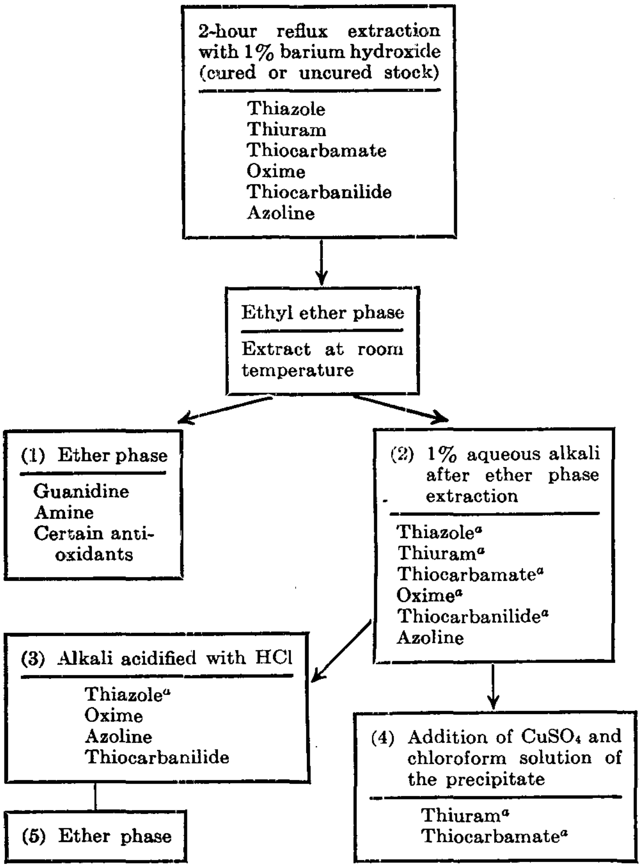
It has been established that guanidines are completely extracted from a compounded, cured stock in as little time as 2 hours (*12*), with a much shorter period being required for a qualitative check. Qualitative extraction of other acid- and alkali-soluble accelerators has been made in about the same time. Size of the sample is not a critical factor, and as little as 0.1 gram of stock may be used for the qualitative detection of thiazoles.

A spectral identification of the prepared "fraction" is made using a Beckman quartz ultraviolet spectrophotometer with the usual 1-cm. quartz cells. Most organic accelerators will show some selective absorption in the 220- to 380-mμ region, when the accelerator is dissolved in a suitable solvent (Table III).

Familiarity with the techniques and the spectral curves of the various accelerator fractions from selective solvent extractions enables an analyst to complete a survey for all of the five classes of accelerators in approximately 4 hours. This time could be shortened considerably if a recording instrument were available.

Although this paper is concerned only with qualitative analysis, most of the accelerators mentioned have been quantitatively recovered from different types of cured or uncured stocks. The authors plan to publish quantitative techniques utilizing these basic qualitative procedures.

Table II. Outline B. Procedure for Accelerator Identification with Alkali Extract



[a] Major or most reliable test. Other tests are more or less confirmatory and are not always needed for identification.

EXTRACTION PROCEDURE

Alkali. The stock to be used for analysis is thoroughly milled and sheeted thinly in a cool mill, and 1 to 2 grams are added to a ground-glass–jointed Erlenmeyer flask. The sample is refluxed in about 50 ml. of 1% barium hydroxide, with the flask connected to a condenser fitted with a ground-glass tapered joint. The hot solution, when filtered and cooled, is ready for the spectral analysis indicated in Table II.

Before a spectral analysis is made, the solution should be liquid-liquid extracted with chloroform or ethyl ether to remove small amounts of dissolved antioxidants and softeners, which are known to be present in the hot alkaline solution but which are only slightly soluble on cooling. Guanidines may also be qualitatively identified in this ethyl ether extract, if they are present alone or in large quantities.

Acid. The procedure is identical to that for the alkali extraction, with the exception of the solvent. Here approximately 50 ml. of 1% hydrochloric acid are used if the stock is cured and an additional 50 ml. of benzene if an uncured stock is being analyzed. If benzene has been used, the benzene is removed after cooling and the acid solution boiled for a few minutes to remove any traces of benzene. At this stage the acid solution is ready for any special preparation which may be required for the complete analysis.

Thiazoles will be extracted from the aqueous acid by the initial ethyl ether phase extraction, but as they are best determined in the alkaline extract, they may be considered as interference here.

AQUEOUS ALKALI EXTRACT

Aromatic Thiazoles. Aromatic thiazoles are characterized by a maximum found in the alkali at 308 mμ. This is the barium salt of mercaptobenzothiazole. Substituted thiazoles, such as *N*-oxydiethylene benzothiazole-2-sulfenamide (Nobs) or benzothiazyl-2-monocyclohexylsulfenamide (Santocure) will exhibit this same maximum, for the alkali causes the molecule to split and yields the characteristic thiazole salt. When the alkaline solution is made acid to litmus, the maximum shifts to 322 mμ (Figure 1), which represents the absorption associated with mercaptobenzothiazole itself.

Aliphatic Thiazoles. The aliphatic thiazoles, Texas and Erie, which are mixed ethyl- and dimethylmercaptothiazoles, also exhibit a gradual shift in location of absorbance maximum as the pH is varied. Texas, in a solution alkaline to litmus, shows a relatively symmetrical maximum at 298 mμ with a shift to 318 mμ when the solution is made acid to litmus. Erie in alkaline solution has a maximum at 294 mμ, and, when acidified, the maximum is located at 314 mμ. The 4- and 14-mμ difference in location of maxima in acid and base serves to differentiate the aromatic and aliphatic thiazoles.

The presence of a thiazole is further confirmed by acidifying the aqueous alkaline solution with concentrated hydrochloric acid and extracting with ethyl ether. The ether phase will give a spectral curve similar to that in aqueous acid, but with the maximum at 326 mμ. This is the normal absorption curve for 2-mercaptobenzothiazole in an organic solvent.

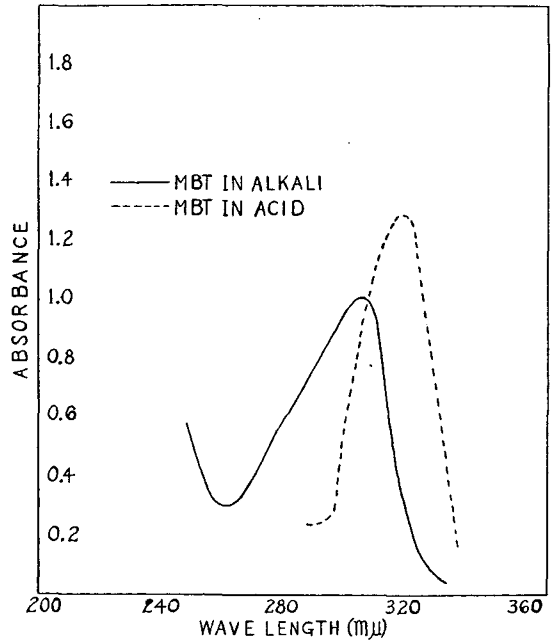


Figure 1. Thiazoles in aqueous barium hydroxide and hydrochloride acid solutions

Because of the formation of 2,2′-benzothiazyl disulfide when 2-mercaptobenzothiazole is used in a compounding formula, or vice versa, depending on the conditions of vulcanization, it cannot be determined which aromatic thiazole was added. It is also known that zinc salts of the thiazoles may be formed under the conditions of vulcanization where zinc oxide is present, if either 2,2′-benzothiazyl disulfide or 2-mercaptobenzothiazole has been used. Here, the thiazole will be found as 2-mercaptobenzothiazole alone, and not as the individual 2,2′-benzothiazyl disulfide or zinc mercaptobenzothiazole.

Thiocarbamates. The alkaline solution will contain added thiocarbamates and any thiocarbamates which have been formed by the action of zinc oxide and thiurams during vulcanization, or during the extraction by the alkali itself. Absorbance of thiocarbamates in aqueous alkali (Figure 2) is as characteristic as that of the copper salt in chloroform, but is subject to greater interference from other alkali-soluble accelerators, particularly thiazoles. If thiazoles are absent from a stock, then identification can be made in the alkali alone without resorting to formation of the copper complex.

Positive identification of the type of thiocarbamate can be made here by precipitating any thiocarbamate with 1% copper sulfate solution. The resulting deep brown copper thiocarbamate precipitate, soluble in chloroform, is a positive test for the presence of the thiocarbamate. (If a brown precipitate is formed which is insoluble in chloroform, free sulfur is most probably present in the stock in rather large quantities.) The thiocarbamate is identified by a spectral check of the copper complex in chloroform (Figure 3). Here distinct double maxima are found at positions dependent upon the structure of the thiocarbamate formed. The location of the maximum at the shortest wave length increases altogether about 8 mμ, and the prominence of the secondary maximum is reduced as the molecular weight of the attached group increases through methyl, ethyl, butyl, and benzyl groups.

Thiocarbamates also react with many ions other than copper to yield white or colored complexes which show selective absorbance in the ultraviolet region. Nickel or cobalt salts give a thiocarbamate with a spectral curve distinctly different from that of the copper complex. The difference is so pronounced that sodium diethyldithiocarbamate has been used for spectrophotometric detection of copper, cobalt, and nickel simultaneously (*4*). However, copper appears to be as good as any metallic ion tested for the purpose of distinguishing the different thiocarbamates.

Thiurams. Thiurams are normally extracted by the aqueous alkali as the thiocarbamate derivative. It is not possible to distinguish tetramethylthiuram monosulfide from tetramethylthiuram disulfide in a cured stock, as both are normally converted partially or almost completely to their zinc thiocarbamate salts. The absorbance curve of tetramethylthiuram monosulfide itself in an organic solvent is distinctly different from that of tetramethylthiuram disulfide, and identification is simple if the accelerators are recovered in their original form. If the stock is uncured, much of the soluble tetramethylthiuram monosulfide may be extracted with distilled water, in which tetramethylthiuram disulfide is essentially insoluble. As with thiocarbamate accelerators, the copper complex will distinguish between ethyl and methyl thiurams.

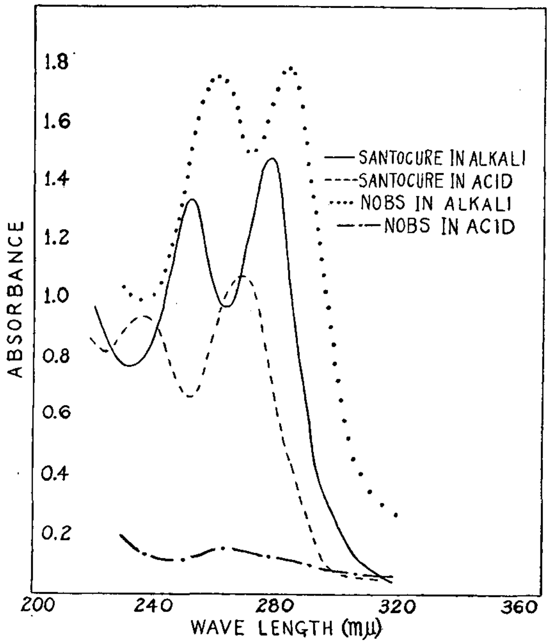


Figure 2. Thiocarbamate derivatives of sulfenamides in aqueous sodium hydroxide and hydrochloric acid

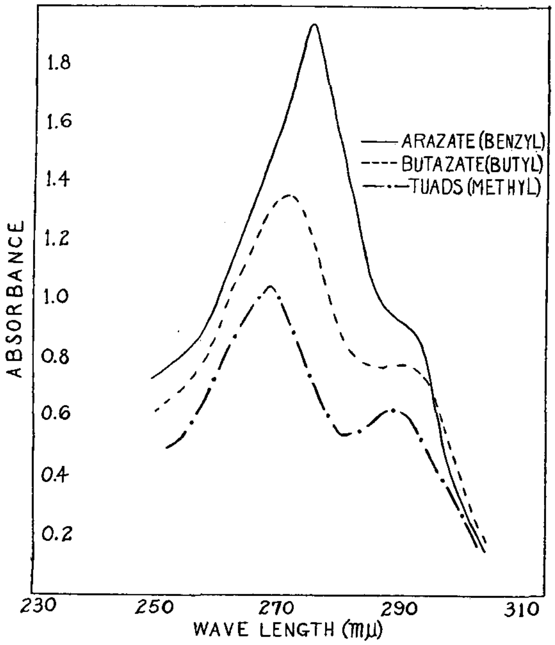


Figure 3. Copper complex of thiurams and thiocarbamates in chloroform

AQUEOUS ACID EXTRACT

Guanidines. The guanidines, being alkaline materials, are found in the acid phase after extraction with ethyl ether to remove any thiazole. The curve of the guanidine hydrochloride in acid is characteristic for triphenylguanidine, diphenylguanidine, and di-*o*-tolylguanidine (Figure 4). Confirmatory identification of the type of guanidine is carried out by making the acid extract strongly alkaline to litmus with ammonium hydroxide and extracting this alkaline solution with ethyl ether. Each of the three types of guanidines mentioned here will give a characteristic single maximum which is the same as that of the individual guanidine accelerator in an organic solvent.

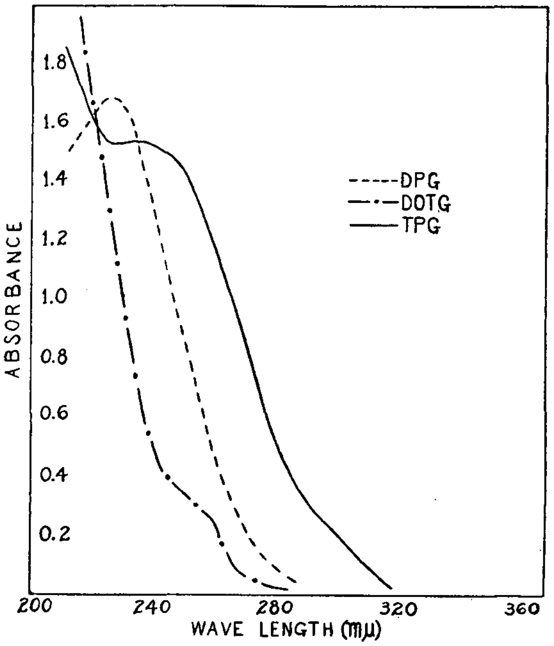


Figure 4. Guanidines in aqueous 1% hydrochloric acid

It is also possible to determine the presence of a guanidine material in such compounds as diphenylguanidine oxalate and diphenylguanidine phthalate, which are substituted diphenylguanidines. The guanidine component of the molecule is normally determined as such.

Guanidines are easily separated from thiazoles in stocks where they occur together by the preliminary ethyl ether extract of the aqueous acid stock extract. It is advisable to extract the acid phase two to three times with ethyl ether to remove the last traces of any thiazole.

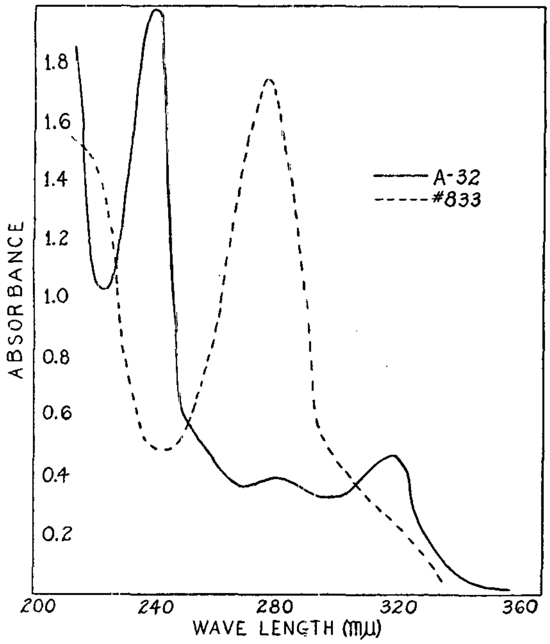


Figure 5. Aldehyde-amines in aqueous hydrochloric acid

Sulfenamides. The presence of a substituted thiazole or sulfenamide, such as *N*-oxydiethylene benzothiazole-2-sulfenamide or benzothiazyl-2-monocyclohexylsulfenamide, can be detected by identifying the amine associated with this compound. Here the acid breaks up the molecule to form a thiazole and an amine hydrochloride. Since the amine itself does not absorb selectively, it is treated with carbon disulfide to produce a thiocarbamate which exhibits characteristic ultraviolet absorption. The amine present in the acid extract of a stock can be removed by making the acid extract strongly alkaline to litmus with sodium hydroxide and extracting with chloroform. The chloroform is evaporated carefully on a water bath to less than 5-ml. volume. To this are added one pellet of solid reagent sodium hydroxide, an equal volume of carbon disulfide, and a few pieces of coarse silica gel to ensure as anhydrous a solution as possible. (The preparation should be dry to prevent formation of interfering carbon disulfide absorbance at 308 to 312 mμ attributed to xanthate formation.) The mixture is evaporated to dryness on a water bath and the residue dissolved in water. Ultraviolet absorbance of the aqueous alkali is then recorded. It will show characteristic absorbance of the sodium salt of the particular thiocarbamate present.

Confirmation of the type of sulfenamide present can be obtained by acidifying this alkaline solution. If benzothiazyl-2-monocyclohexylsulfenamide is present, two maxima are observed at approximately the positions of the two minima in the alkaline solution (Figure 2). *N*-oxydiethylene benzothiazole-2-sulfenamide presents a spectral curve in the alkaline solution similar to benzothiazyl-2-monocyclohexylsulfenamide, but with maxima at different wave lengths. Making the solution acid to litmus will destroy any strongly selective absorbing material, thus differentiating *N*-oxydiethylene benzothiazole-2-sulfenamide from benzothiazyl-2-monocyclohexylsulfenamide. The absorbance of the acidified solution is unstable and should be recorded immediately.

If copper sulfate is added to a part of the alkaline solution, a brown precipitate soluble in chloroform is further proof of the presence of thiocarbamates. Spectral absorbance here will aid in identification of an amine added as such originally. Any amine residue from thiocarbamates or thiurams added to the stock and decomposed by the acid may be expected to appear here. However, absorbance characteristics are sufficiently different so that sulfenamides may be identified by this procedure.

Aldehyde-Amines. Aldehyde-amines will be found in the aqueous acid extract of a cured, compounded stock after the initial ethyl ether extraction. If aromatic amines and certain aliphatic aldehyde-amines are present, the spectral curves in aqueous acid are usually characteristic enough for identification, where thiazoles and guanidines are absent (Figure 5). If thiazoles are present, they will be removed by ethyl ether extraction, leaving the characteristic absorbing material associated with the amine in the acid extract. By following the outlined procedure and preparing thiocarbamate derivatives, the common aliphatic amines may also be detected. It should be noted that sulfur vulcanization of a rubber compound with an aldehyde-amine may yield a trace of a thiocarbamate residue. If an aldehyde-amine is detected as such in considerable quantity, and only a trace of a thiocarbamate is present, it is probable that the thiocarbamate was formed from the amine during vulcanization.

SPECIAL PURPOSE ACCELERATORS

There are a number of special purpose accelerators which can be detected in the alkali and acid extracts of cured compounded stocks.

Oximes. A stock accelerated with *p,p'*-dibenzoquinonedioxime shows a maximum in alkali at 361 mμ, while the maximum in the same solvent for *p*-quinonedioxime is found at 317 mμ.

Azolines. The presence of 2-mercaptothiazoline can be detected by the sharp maximum found in alkali at 246 mμ, with a "shoulder" at 264 mμ. Acidification of the alkaline solution gives a single symmetrical maximum at 268 mμ. 2-Mercaptoimidazoline can be differentiated from 2-mercaptothiazoline by the single maximum in alkali at 232 mμ. No change is observed when this solution is acidified.

Thiocarbanilide. This exhibits a broad maximum at 252 mμ in alkali and in the alkali extract after it is made acid. Ethyl ether phase extraction of the acid gives the normal single 280-mμ maximum of this material in an organic solvent.

Xanthates. Little work has been done with xanthates, but the commercial compound dibutylxanthogen disulfide exhibits selective absorption in alkali solution with maxima at 226 and 302 mμ. If the xanthates do not decompose completely during curing, it is believed that some qualitative detection could be made. Identification would be more certain if an uncured sample were used.

Several other special purpose accelerators not mentioned here will give characteristic absorbance curves at some point, using the method outlined. Any new accelerators which are acidic or alkaline in nature may be expected to fit into this scheme of analysis.

RETARDERS

Retarders are sometimes added to prevent premature vulcanization of a stock. They are usually acid in nature and can be detected along with accelerators. Salicylic acid could be considered as interference in the detection of guanidines and thiazoles with the hydrochloric acid extract. The characteristic double maxima of salicylic acid at 236 and 306 mμ are observed in the aqueous acid itself, when thiazoles are absent. When present, the thiazoles are removed by first adjusting the pH of the solution to between 8.5 and 9.0. It has been determined that thiazoles are completely extracted with ethyl ether in this pH range, but that salicylic acid is retained in the aqueous alkali. Salicylic acid itself can be identified by again making the alkaline solution acid to litmus with hydrochloric acid and liquid-liquid extracting the salicylic acid with ethyl ether. Phthalic anhydride (changed to phthalic acid by the action of the solvent) may be detected in a similar manner and is readily distinguished from salicylic acid by its absorbance curve in aqueous acid solution.

Table III. Absorbance Characteristics of Accelerator Fractions

Trade Names	Chemical Names	Index No./Solvent[a]	Wave Length of Maxima, mμ[b]	Absorbance[c] Ratio
Aromatic Thiazoles				
MBT, Captax, Thiotax	2-Mercaptobenzothiazole	A1/C; B5/E	329 236	329/274 = 14 329/236 = 1.8
		B2/B	308 232	232/308 = 1.1
		B3/A	322 232	322/232 = 1.8
MBTS, Altax, Thiofide	2,2′-Benzothiazyl disulfide		Identical with MBT	
Santocure	Benzothiazyl-2-monocyclohexylsulfenamide		Thiazole portion identical with MBT	
		A4/B[d]	284, 252	284/252 = 1.1
		A6/A	270, 236	270/236 = 1.1
Nobs, Amax	*N*-Oxydiethylene benzothiazole-2-sulfenamide	A4/B[d]	286, 262	286/262 = 1.0
		A6/A	No maxima	
OXAF, Zenite Special	Zinc benzothiazyl sulfide		Identical with MBT	
Aliphatic Thiazoles				
Texas	Mixed ethyl and dimethyl mercaptothiazoles	B5/C	326	326/276 = 16
		B2/B	298	298/248 = 4.6
		B3/A	318	318/266 = 9.5
Erie	Mixed ethyl and dimethyl thiazyl disulfides	B2/B	294	294/242 = 3.2
		B3/A	314	314/246 = 3.8
Guanidines				
DPG	Diphenylguanidine	B1/E		Identical with A3/E
		A2/A	226	226/246 = 1.7
		A3/E	258	258/278 = 1.6
DOTG	Di-*o*-tolylguanidine	B1/E		Identical with A3/E
		A2/A	254 (shoulder)	254/274 = 9.3
		A3/E	254	254/274 = 1.9
TPG	Triphenylguanidine	B1/E		Identical with A3/E
		A2/A	235	235/226 = 1.02
		A3/E	263	263/284 = 1.4
Permalux	Di-*o*-tolylguanidine salt of dicatechol borate	B2/B	276	276/250 = 2.8
		A2/A	274	274/254 = 1.8
		A3/E	254	254/274 = 1.9
Thiocarbamates				
Methyl Zimate, Methazate	Zinc dimethyldithiocarbamate	B2/B B4/C		Identical with methyl Tuads
Ethyl Zimate, Ethazate	Zinc diethyldithiocarbamate	B2/B B4/C		Identical with ethyl Tuads
Butyl Zimate, Butazate	Zinc dibutyldithiocarbamate	B2/B	258, 282	258/282 = 1.0
		B4/C	274, 290	274/290 = 1.7
Arazate	Zinc dibenzyldithiocarbamate	B2/B	258, 290	258/290 = 1.1
		B4/C	276, 294	276/294 = 2.1
Selenac	Selenium diethyldithiocarbamate	B2/B B4/C		Identical with ethyl Tuads
Pip-Pip	Piperidine pentamethylenedithiocarbamate	B2/B	258, 282	258/282 = 1.3
Thiurams				
TMTD, Methyl Tuads, Tuex	Tetramethylthiuram disulfide	B2/B	252, 278	252/278 = 1.0
		B4/C	268, 290	268/290 = 1.6
TETD, Ethyl Tuads	Tetraethylthiuram disulfide	B2/B	258, 282	258/282 = 1.0
		B4/C	272, 290	272/290 = 1.6
Monex, Thionex	Tetramethylthiuram monosulfide	B2/B		Identical with methyl Tuads
Aldehyde-Amines				
A-32, 808	Butyraldehyde and aniline reaction product	B4/C		
		A2/A	242, 322, 280	242/322 = 4.3
		B2/B	228, 280	228/280 = 2.2
Accelerator 833	Butyraldehyde-monobutylamine reaction product	A2/A	278	278/242 = 3.7
		B2/B	278	278/244 = 5.6
Trimene base	Triethyltrimethylenetriamine	A2/A	242, 324	242/324 = 3.7
		B2/B	268, 298	268/298 = 1.3
A-19	Acetaldehyde-formaldehyde-aniline product	A2/A	237, 314	237/314 = 2.9
Azolines				
2-MT	2-Mercaptothiazoline	A2/A	268	268/230 = 5.0
		A1/E, A3/E	268, 248	268/248 = 2.3
		B2/B	246, 264	246/264 = 1.1
		B3/A	268	268/230 = 5.1
NA-22	2-Mercaptoimidazoline	A2/A	268	268/230 = 4.6
		A1/E, A3/E	278, 248	278/248 = 2.2
		B2/B	232	232/252 = 11.3
		B3/A	232	232/252 = 6.2
Special purpose				
Thiocarbanilide		A3/E	240, 288	240/288 = 4.3
		B2/B, B3/A	252	252/272 = 1.4
		B5/E	280	280/246 = 2.3
Aniline		A2/A	240 280	240/280 = 6.1
Vulcanizing agents				
Dibenzo GMF	*p,p′*-Dibenzoquinonedioxime	B2/B	360	360/380 = 3.5
		B3/A	314	314/254 = 2.0
		B5/E	313	313/334 = 2.6
GMF	*p*-Quinonedioxime	B2/B	317	317/336 = 2.0
		B3/A	316	316/336 = 1.9
		B5/E	314	314/334 = 2.7
Activators				
Barak	Dibutylammonium oleate	A4/B[d]	258, 282	258/282 = 1.1
		A5/C	274	274/290 = 1.7
DBA	Dibenzylamine	A4/B[d]	258, 284	258/284 = 1.1
		A5/C	276, 294	276/294 = 1.9
Retarders				
Retarder W	Salicylic acid	A2/A	236, 304	236/304 = 2.2
		A1/E	236, 306	236/306 = 1.8
E-S-E-N	Phthalic anhydride	A2/A	230, 280	230/280 = 5.5
		A1/E	224, 276	224/276 = 6.4

[a] Index No. refers to procedure for accelerator identification—either Outline A (acid) or Outline B (base) with the number representing a given block within each outline. Thus, A3/ indicates acid outline, block 3. For the solvent, the following abbreviations apply: A, aqueous acid; B, aqueous alkali; E, ethyl ether; and C, chloroform. Chloroform and ether may usually be used interchangeably. Where chloroform is used in place of ether, the wave length of the maximum will be shifted about 2 mμ toward the longer wave-length region.

[b] Primary or most characteristic maximum listed first. In a few cases the secondary maximum is no more than a "shoulder," but it is characteristic.

[c] Ratio of absorbance of strongest and next most intense maximum where two maxima are present. Represents ratio of maximum to adjacent minimum where single maximum is present. If no minimum is present, ratio is that of maximum absorbance to an arbitrarily selected wave length 20 mμ toward longer wave-length region. Purpose of ratio is to indicate selectivity or sharpness of maximum, which is important for identification. Ratio given is believed reasonably accurate, but may be expected to vary somewhat depending on purity and amount of interference in extract.

[d] Barium salts are represented in all alkaline solutions except for (A4/B)[d], where absorbance reported is that of sodium salt. Prepared thiocarbamate derivatives of sulfenamides and amine activators are sodium salts.

ACTIVATORS

Some of the compounding formulas in use in the rubber industry at the present time employ the use of an organic activator of the amine type. If present in a stock, these will be extracted with 1% hydrochloric acid and their presence can be determined

▼

following Table I with particular attention to the blocks concerning the identification of amines.

DISCUSSION

Selective solvent extraction coupled with ultraviolet spectrophotometry offers a strong tool for the identification of many alkaline and acidic organic materials when present in trace amounts in natural or commercial products.

It is generally agreed (*14*) that ultraviolet spectrophotometry is more sensitive than infrared. This is of particular value in identification of trace amounts of rubber compounding materials such as accelerators and antioxidants, which are usually put into a compounding formula in quantities of 1% or less.

The infrared may be more specific than the ultraviolet in distinguishing between similar accelerators. However, it has been shown here that similar compounds can often be converted by simple chemical treatment to give distinctly different ultraviolet absorbance characteristics which will readily identify them. The short spectral range of the ultraviolet also makes for more rapid identification. Where applicable, the 0.12-micron range of 220 to 340 mμ in the ultraviolet will give as positive identification of most common rubber accelerators as will complete coverage of the 1- to 14-micron range in the infrared.

The tedious chromatographic isolation of accelerators will usually recover the accelerator as the compound or compounds present in the stock. This has both advantages and disadvantages. Chromatography coupled with ultraviolet and infrared spectroscopy may be the ideal method for identification of accelerator reaction products in a cured stock. Many accelerators are converted partially or entirely during vulcanization to different compounds. Therefore, identification of a single added accelerator by chromatography may mean isolation of two to four compounds which might confuse matters as to which material was added originally. Thus a tetramethylthiuram disulfide cured stock may have tetramethylthiuram disulfide, zinc dimethyldithiocarbamate, or tetramethylthiuram monosulfide present. A benzothiazyl-2-monocyclohexylsulfenamide cured stock may have the original benzothiazyl-2-monocyclohexylsulfenamide, 2-mercaptobenzothiazole, 2,2′-benzothiazyl disulfide, and traces of cyclohexylamine or thiocarbamate formed from the cyclohexylamine. Aldehyde-amine accelerators may leave traces of thiocarbamates on curing.

The rapid selective solvent extraction of accelerators outlined here will often convert accelerator breakdown products in a cured stock to a single material of known composition. This fact, and the use of aqueous solutions to eliminate interference of such materials as softeners, presents a practical procedure for the identification of general classes of accelerators.

Though barium hydroxide is used here, the solvent could just as well be sodium or potassium hydroxide, or sodium sulfite in many cases. Also, ethyl ether used for some phase extractions and as a solvent for ultraviolet absorbance might be replaced by chloroform. Chloroform is a stronger solvent and is nonflammable, but ether is usually preferred because chloroform does not transmit ultraviolet below 246 mμ, whereas ethyl ether transmits to about 220 mμ.

One factor working in the analyst's favor is the fact that accelerators which might possibly interfere with each other in the proposed method of analysis are rarely used in the same rubber compound. Thus, thiocarbamate and aldehyde-amine accelerators are both considered fast curing and are not commonly used together in rubber products. As sulfenamides are used primarily where scorching is a problem, no ultra accelerators such as amines or thiocarbamates will be used in the same stock. Also, common practice dictates that where more than one accelerator is used, a combination of an acidic thiazole with an alkaline guanidine, thiuram, or amine is normal procedure.

Therefore, this method of selective solvent extraction to separate alkaline and acid materials fits in well with present-day rubber compounding practices.

ACKNOWLEDGMENT

The authors gratefully acknowledge the permission of the Firestone Tire and Rubber Co. to publish this article. The helpful assistance of George H. Wallace in preparing the final manuscript, of Marlene Brey for some of the spectrophotometric work, and of Burrell Larimore for preparing the figures is greatly appreciated.

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Received for review September 27, 1954. Accepted December 16, 1954. Presented before the Division of Rubber Chemistry at the 126th Meeting of the American Chemical Society, New York, September 1954.

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Chelation of Platinum Group Metals

Complexometric Titration of Palladium

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Palladous solutions can be titrated complexometrically by addition of excess ethylenediaminetetraacetic acid followed by back-titration with zinc nitrate. The pH is controlled at 10 ± 1 and Eriochrome Black T is used as an indicator. The titration has been applied to 0.6 to 30 mg. of palladium. Bivalent and quadrivalent platinum do not interfere. Iridium and ruthenium interfere partially at higher concentrations. The titration is not applicable in the presence of rhodium or osmium.

THE spectrophotometric determination of palladium has been discussed (*8*). It depends upon the formation of a yellow complex between ethylenediaminetetraacetic acid and palladous ion. In a continuation of the general study of the chelation of the platinum group metals with ethylenediaminetetraacetic acid, it has been found that the formation of this complex may be used as the basis for a volumetric determination of palladium.

Previous titrations of palladium have involved titration with iodide (*1, 7*), addition of excess salicylaldoxime followed by oxidimetric titration of excess reagent (*4*), oxidimetric titration with ferrous sulfate and ceric sulfate (*12*), addition of β-furfuraldoxime followed by oxidimetric back-titration with ceric sulfate (*13*), and the acidimetric titration of palladium based on the liberation of hydroxyl ions from the reaction between the palladous cyanide complex, $Pd(CN)_4^{--}$, and excess mercuric oxide (*2*). Flaschka (*3*) has also reported the use of ethylenediaminetetraacetic acid in a titration of palladium in which an excess of nickel cyanide is added and the displaced nickel is then titrated with ethylenediaminetetraacetic acid. Direct complexometric titrations for a number of ions using the reagent ethylenediaminetetraacetic acid have been reported by Schwarzenbach and coworkers (*9–11*). The direct titration of palladous ion with ethylenediaminetetraacetic acid is not applicable in the presence of chloride ions because there is not enough free palladous ion to produce a satisfactory color change with the indicator Eriochrome Black T. In addition, palladous hydroxide precipitates at the pH necessary for the proper functioning of this indicator. Harris and Sweet (*5*) have modified this procedure by adding an excess of ethylenediaminetetraacetic acid to the metal ion and then back-titrating the excess with zinc nitrate using Eriochrome Black T as indicator.

It has been found that the Harris and Sweet modification can be applied to the complexometric titration of palladium. It is applicable to macro and micro quantities of palladium and the precision is better than that claimed by previous authors. Bivalent and quadrivalent platinum do not interfere but iridium and ruthenium interfere at high concentrations.

The analytical results obtained in analyses with known amounts of palladium are shown in Table I. The average of the errors in six sets of triplicate determinations ranging from 0.6 to 30 mg. of palladium is 0.2%.

The pH of the palladium solution was regulated at 10 ± 1 by the addition of dilute potassium hydroxide so that the indicator Eriochrome Black T would function properly. Buffers were not satisfactory for the control of pH because of the tendency of palladium to form strong complexes with the buffer materials.

In general, best results were obtained when the excess of ethylenediaminetetraacetic acid was kept small. In the more concentrated solutions of palladium, the yellow color of the palladium–ethylenediaminetetraacetic acid complex tends to obscure the color of the indicator and a large amount of Eriochrome Black T must be added in order to make the end point visible. At lower concentrations the color change of Eriochrome Black T is blue to pink while at higher concentrations, the color change is from yellow-green to pink. Chloride ion concentrations of 0.01 to 1.0*M* had no effect upon the titration.

The interference of other platinum group metals was studied by adding known amounts of hydrochloric acid solutions of osmium(IV), ruthenium(III), platinum(II), platinum(IV), rhodium(III), and iridium(IV) to the standard palladium solutions. An excess of ethylenediaminetetraacetic acid was added, the pH adjusted to 10, Eriochrome Black T added, and the solutions were immediately back-titrated with the standard zinc solution. In all cases, the solutions containing other platinum metals were

Table I. Complexometric Titration of Palladium

Pd Taken, Mg.	Pd Found		Error in Mean	
	Mg.	Mean	Mg.	%
30.67	30.58 30.65 30.53	30.59	−0.08	0.3
21.42	21.32 21.37 21.51	21.40	−0.02	0.1
15.21	15.25 15.27 15.23	15.25	+0.04	0.3
6.128	6.136 6.132 6.136	6.135	+0.007	0.1
3.064	3.063 3.061 3.059	3.061	−0.003	0.1
0.620	0.621 0.622 0.621	0.621	+0.001	0.2
			Average error	0.2%

Table II. Effect of Other Platinum Metals on Complexometric Titration of Palladium

Pd Taken, Mg.	Metal Added, Mg.	Pd Found		Error in Mean	
		Mg.	Mean	Mg.	%
3.064	4.7 (Pt^{IV})	3.057 3.059 3.062	3.060	−0.004	0.1
3.064	4.0 (Pt^{II})	3.063 3.060 3.059	3.061	−0.003	0.1
3.064	2.0 (Ir^{IV})	3.099 3.090 3.094	3.094	+0.030	1.0
3.064	1.0 (Ru^{III})	3.146 3.144 3.134	3.139	+0.075	2.4

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treated in a manner identical with that used for those containing only palladium. The results are shown in Table II. The chloride complexes of bivalent and quadrivalent platinum are sufficiently stable to prevent the reaction of these ions with ethylenediaminetetraacetic acid under these conditions, hence, platinum does not interfere with the complexometric determination of palladium. However, if the titration is made at room temperature, and as rapidly as possible after the ethylenediaminetetraacetic acid is added, ruthenium or iridium does not interfere seriously.

The rhodium chloride complex is sufficiently strong to prevent the formation of a rhodium–ethylenediaminetetraacetic acid complex in chloride solution. However, the presence of rhodium seriously interferes in another way with the complexometric titration of palladium. The effect of the rhodium is to increase the amount of zinc solution required for a back-titration, thereby decreasing the apparent concentration of palladium by as much as 50%. The nature of the reaction is not known.

Chloride solutions of palladium containing even small amounts of osmium inactivate and decolorize several milliliters of Eriochrome Black T, making the end point almost impossible to detect. It was found that palladium solutions containing more than a trace of osmium could not be satisfactorily titrated complexometrically using Eriochrome Black T as an indicator.

RECOMMENDED PROCEDURE

Add a slight excess of a standard solution of the disodium salt of ethylenediaminetetraacetic acid to a chloride solution of bivalent palladium. Regulate the pH to 10 ± 1 with 0.1N potassium hydroxide. Add 5 drops of the Eriochrome Black T indicator solution and titrate with a standard zinc solution until the equivalence point is reached, as shown by the color change from blue or green to pink.

REAGENTS

Standard Palladium Solution. A 2.5-gram sample of palladous chloride, from Coleman and Bell Co., was dissolved in 500 ml. of 0.2M hydrochloric acid. Spectrographic investigation showed only traces of platinum present. This solution was standardized by precipitation of the palladium with dimethylglyoxime followed by drying at 110° C. (*6*).

Standard Zinc Solution. A 1.8-gram sample of Mallinckrodt analytical reagent grade zinc oxide was dried for 2 hours at 100° C., dissolved in a minimum amount of 1 to 1 nitric acid, and diluted to 1 liter with distilled water. This solution was standardized by precipitating with ammonium phosphate and weighing the zinc as the pyrophosphate according to the procedure of Vance and Borup (*14*).

Standard Ethylenediaminetetraacetic Acid Solution. A 5.5-gram sample of the disodium salt of ethylenediaminetetraacetic acid, analytical reagent grade from Versenes, Inc., was dissolved in 1 liter of distilled water. The molarity of this solution was obtained by a complexometric titration using Eriochrome Black T and the standard zinc solution.

Indicator Solution. A 0.1-gram sample of Eriochrome Black T from W. H. and L. D. Betz Co. was dissolved in 50 ml. of distilled water which had been made slightly basic with 3 drops of 1N potassium hydroxide.

Other Platinum Metals. Reagent grade perosmic acid, ruthenium chloride, platinous chloride, platinic chloride, rhodium chloride, and iridium chloride were used for the study of interferences. Spectrographic analysis showed less than significant amounts of impurities. All solutions were standardized, using modifications of the Gilchrist-Wichers scheme (*6*).

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RECEIVED for review August 9, 1954. Accepted December 7, 1954.

Action of *N*-Bromosuccinimide on Ascorbic Acid

New Titrimetric Method for Estimation of Vitamin C

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Estimation of ascorbic acid with 2,6-dichlorophenolindophenol is limited by the presence of interfering substances such as reductones and reductic acid. Slow reducing substances that may be present in biological fluids render the end point less distinct. This defect induced the authors to investigate the estimation of ascorbic acid by *N*-bromosuccinimide, the oxidizing action of which might be selective. The fact that ascorbic acid is selectively oxidized by *N*-bromosuccinimide before other reducing substances that may be present provides a reliable titrimetric method of extensive applicability. The proposed method is simple, rapid, and sufficiently sensitive to determine a concentration as low as 7.04 γ of ascorbic acid per milliliter. The experimental error does not exceed ±2%.

A VARIETY of bioassay and chemical methods for the determination of vitamin C activity or ascorbic acid content has been developed (*4, 11, 12, 17*). Although bioassays have the advantage of measuring the summation of chemical entities that possess vitamin C activity, they are time-consuming and expensive, and leave much to be desired in precision. Consequently, bioassays are now confined to use in comparative studies to establish the specificity of chemical methods for determining ascorbic acid in individual products.

Chemical methods for the determination of vitamin C are based mainly upon the reducing properties of the vitamin. These methods include titration of an acid extract with iodine, methylene blue, ferricyanide, and 2,6-dichlorophenolindophenol (*9, 19, 20*). Oxidation of ascorbic acid with the dye 2,6-dichlorophenolindophenol is the most satisfactory and extensively used method (*5, 6*). However, the value of the 2,6-dichlorophenolindophenol reagent for measuring ascorbic acid is limited by the presence of other reducing substances such as reductones, reductic acid, and dihydroxymaleic acid, which give rise to errors, particularly in food products. Inorganic and organic ferrous and ferric compounds also interfere with the determination by 2,6-dichlorophenolindophenol (*3*, *13*, *18*). Ferrous ion reduces the dye in the presence of metaphosphoric acid, so that pharmaceutical preparations containing reduced iron should be titrated in 8% acetic acid solution free from metaphosphoric acid. On the other hand, ferric ion interferes with the end point in the absence of metaphosphoric acid, so that metaphosphoric–acetic acid mixture should be employed as the titration medium in testing pharmaceutical preparations containing oxidized iron (*10*).

The end point with plant tissues is stable for some time, but many animal tissue extracts and urines contain sufficient amounts of slow-reducing substances to make the end point less distinct, especially when small quantities of ascorbic acid are being titrated. In such cases the accuracy of the titration depends on the skill of the observer in detecting the point at which rapid reduction is replaced by a slow fading of the indicator.

Colorimetric methods for estimating ascorbic acid, which are not based upon oxidation-reduction properties, include the 2,4-dinitrophenylhydrazine method (*14–16*), which is too tedious and time-consuming for routine analyses. The photoelectric colorimetric method for the determination of ascorbic acid, by means of uranium nitrate (*1*), has been applied only to test solutions and pharmaceutical products.

A new easy, rapid, and accurate titrimetric method for the estimation of ascorbic acid in pure solutions, pharmaceutical preparations, biological fluids—e.g., blood and urine—and edible fruits, which overcomes the disadvantages previously mentioned, is based on the selective oxidation of ascorbic acid by *N*-bromosuccinimide.

EXPERIMENTAL

Equipment and Reagents. A microburet of 5-ml. capacity, graduated in hundredths of a milliliter.

Graduated pipets of 1-, 2-, and 5-ml. capacity.

Erlenmeyer flasks of 25-, 50-, and 100-ml. capacity.

Four per cent weight per volume of potassium iodide in distilled water.

Three per cent volume per volume of acetic acid.

One per cent starch solution prepared by dissolving 1 gram of soluble starch in 10 ml. of boiling water and adding to 90 ml. of saturated sodium chloride solution.

A 0.1% weight per volume *N*-bromosuccinimide aqueous solution which is freshly prepared and may be serially diluted 10 or 100 times as required.

Concentration of *N*-bromosuccinimide solution used in titration. 0.1% weight per volume aqueous solution for pure ascorbic acid solutions and pharmaceuticals; 0.01% weight per volume aqueous solution for urine and fruits; 0.001% weight per volume aqueous solution for blood.

Action of *N*-Bromosuccinimide on Ascorbic Acid. Ascorbic acid (1.76 grams, 0.01 mole) was dissolved in distilled water (30 ml.) and *N*-bromosuccinimide (1.78 grams, 0.01 mole) was added to the ascorbic acid aqueous solution. The mixture was refluxed for 15 minutes, when all *N*-bromosuccinimide went into solution. The colorless solution was distilled off in vacuo, and the solid residue was crystallized from benzene giving colorless crystals, with a melting point of 125–6° C., which were proved to be succinimide by comparison of the melting point and mixed melting point with an authentic sample. Yield was 0.7 gram—i.e., 71.42% of the theoretical yield.

In another similar experiment, after the reaction was complete, a small portion of the colorless solution (5 ml.) was treated with dilute nitric acid and 10% silver nitrate solution, when a yellowish white precipitate of silver bromide was deposited, indicating the presence of hydrobromic acid. To the rest of the colorless reaction mixture was added a mixture of phenylhydrazine hydrochloride (5.78 grams, 0.04 mole) and crystalline sodium acetate (5.44 grams, 0.04 mole); the mixture was heated in a boiling water bath for 30 minutes. Deep orange crystals were deposited from the solution while hot. The crystals were filtered off, dried, and recrystallized twice from ethyl acetate, giving uniform orange crystals with a melting point of 218° C. which were proved to be dehydroascorbic acid osazone by comparison with the melting point and mixed melting point of an authentic sample. Yield was 2.42 grams—i.e., 81.92% of the theoretical yield.

VALIDITY OF REACTION FOR QUANTITATIVE ESTIMATION

Before applying the reaction to the estimation of ascorbic acid in pure solutions, pharmaceuticals, biologicals, and fruits, the authors decided to verify the action of *N*-bromosuccinimide on ascorbic acid from a quantitative point of view. It was assumed that 1 molecule of ascorbic acid was oxidized by 1 molecule of *N*-bromosuccinimide and accordingly equimolecular and bimolecular solutions of ascorbic acid and *N*-bromosuccinimide were prepared.

Table I. Titration of Equimolecular Solutions of Ascorbic Acid and *N*-Bromosuccinimide

Ascorbic Acid Soln., Ml. (1 Ml. = 1.76 Mg.)	*N*-Bromosuccinimide Soln., Ml. (1 Ml. = 1.78 Mg.)
1	1.00
2	1.99
3	3.01
4	4.00
5	5.00
10	10.00

Table II. Comparative Analysis by Proposed Method and by 2,6-Dichlorophenolindophenol

Dilution	Ascorbic Acid Soln., Ml.	Content, γ	Found by NBS[a], γ	Error, %	Found by Dye, γ	Error, %
10×, 176 γ ascorbic acid per ml.	5	880	882	0.24	877	0.34
	4	704	704	...	704	...
	3	528	529	0.19	530	0.38
	2	352	352	...	352	...
	1	176	176	...	176	...
100×, 17.6 γ ascorbic acid per ml.	2	35.2	35.1	0.28	35.1	0.28
	1	17.6	17.6	...	17.5	0.56
1000×, 1.76 γ ascorbic acid per ml.	5	8.80	8.80	...	8.80	...

[a] *N*-bromosuccinimide solution.

Ascorbic Acid Solutions. Ascorbic acid (0.176 gram, 1 millimole), A, and ascorbic acid (0.352 gram, 2 millimole), B, were separately dissolved in distilled water and made up to 100 ml. with distilled water in a standard flask. Solution A contained half the number of molecules of ascorbic acid present in solution B.

***N*-Bromosuccinimide Solution.** *N*-Bromosuccinimide (0.178 gram, 1 millimole) was dissolved in hot distilled water, and the aqueous solution was allowed to cool, and made up to 100 ml. with distilled water in a standard flask.

As the molecular weight of ascorbic acid is 176 and that of *N*-bromosuccinimide is 178, it is clear that ascorbic acid solution A contains the same number of molecules as that present in the *N*-bromosuccinimide solution; ascorbic acid solution B contains double that number of molecules.

Each of the two above-mentioned equimolecular solutions of ascorbic acid and *N*-bromosuccinimide (freshly prepared) was serially diluted 10, 100, and 1000 times with distilled water in 100-ml. standard flasks; various concentrations such as 1 ml. of ascorbic acid solution containing 176, 17.6, and 1.76 γ of ascorbic acid were obtained (Table II).

The above dilutions of ascorbic acid solution were estimated simultaneously, with 2,6-dichlorophenolindophenol. The dye was standardized so that 1 ml. was equivalent to 0.117 mg. of ascorbic acid, but in the case of urine 1 ml. of the dye was equivalent to 0.125 mg. of ascorbic acid (Tables II and IX).

When 1 ml. of the ascorbic acid solution diluted 1000 times—i.e., containing 3.52 γ per ml.—was estimated by *N*-bromosuccinimide oxidation, the end point was not clear. Practically it has been found that the reaction between ascorbic acid and *N*-bromosuccinimide proceeds quantitatively in equimolecular concentrations, and that the validity of the values reported (Tables I, II, and III) is also dependent upon that assumption.

Table III. Titration of Bimolecular Solutions of Ascorbic Acid and Molecular *N*-Bromosuccinimide Solution

Dilution	Ascorbic Acid Soln. B, Ml. (1 Ml. = 3.52 Mg.)	Content, Mg.	NBS Used, Ml. (1 Ml. = 1.78 Mg.)	Found, Mg.	Error, %
	1	3.52	2.00	3.52	...
	2	7.04	4.00	7.04	...
	3	10.56	6.00	10.56	...
	4	14.08	8.00	14.08	...
	5	17.60	10.01	17.62	0.05
	10	35.20	20.01	35.22	0.06
10×, 352 γ per ml.	5	1.760	9.99	1.758	0.11
	4	1.408	8.00	1.408	...
	3	1.056	6.00	1.056	...
	2	0.704	4.00	0.704	...
	1	0.352	2.00	0.352	...
100×, 35.2 γ per ml.	2	0.0704	4.00	0.0704	...
	1	0.0352	2.00	0.0352	...
1000×, 3.52 γ per ml.	5	0.0176	10.00	0.0176	...
	2	0.00704	4.00	0.00704	...

Table IV. Estimation of Pure Ascorbic Acid Solutions by 0.1% *N*-Bromosuccinimide Solution

Ascorbic Acid Soln., %	Volume, Ml.	Content, Mg.	0.1% NBS, Ml.	Found, Mg.	Error, %
0.5	1	5	5.05	4.99	0.2
	2	10	10.10	9.98	0.2
0.4	1	4	4.05	4.00	...
	2	8	8.10	8.00	...
0.3	1	3	3.02	2.98	0.66
	2	6	6.05	5.98	0.33
	3	9	9.10	8.99	0.11
0.2	1	2	2.02	1.99	0.5
	2	4	4.05	4.00	...
	3	6	6.06	5.99	0.16
	4	8	8.10	8.00	...
	5	10	10.10	9.98	0.2
0.1	10	10	10.10	9.98	0.2
	9	9	9.06	8.96	0.4
	8	8	8.05	7.96	0.5
	7	7	7.02	6.94	0.85
	6	6	6.02	5.95	0.83
	5	5	5.05	4.99	0.2
	4	4	4.05	4.00	...
	3	3	3.02	2.98	0.66
	2	2	2.00	1.98	1.00
	1	1	1.01	1.00	...

ESTIMATION OF PURE ASCORBIC ACID SOLUTIONS

Procedure. Into a 50-ml. conical Erlenmeyer flask, a known volume of the unknown ascorbic acid solution is introduced—i.e., 1 ml. contains 1 mg. of ascorbic acid. Then 5 ml. of 4% potassium iodide solution, 2 ml. of 3% acetic acid, and 2 drops of starch solution as an indicator are added. The aqueous *N*-bromosuccinimide solution (0.1% weight per volume) is introduced into the microburet and is allowed to run drop by drop, into the ascorbic acid solution, with continuous shaking. The end point is reached when the last drop of the *N*-bromosuccinimide solution added produces a permanent blue color in the ascorbic acid solution (Table IV).

Each of the above two solutions of 0.1% ascorbic acid and 0.1% *N*-bromosuccinimide was diluted 10 and 100· times, so that 1 ml. contained 100 and 10 γ of ascorbic acid, respectively. The estimation was done at the same time by means of 2,6-dichlorophenolindophenol (Table V).

CALCULATION

$$\text{Ascorbic acid content} = V \times C \times \frac{176}{178} \text{ (mg. or } \gamma\text{)}$$

where

V = volume of *N*-bromosuccinimide solution

C = concentration of *N*-bromosuccinimide solution either in milligrams or micrograms

METHOD OF ASSAY

Pharmaceutical Products. INJECTIONS. For the estimation of ascorbic acid in solutions prepared for injection the following method is recommended. A known volume of the solution is diluted with distilled water in a standard flask so that 5 ml. contain 0.5 to 10 mg. of ascorbic acid. Five milliliters of this dilution are accurately measured and introduced into an Erlenmeyer flask of 25- or 50-ml. capacity. Then 5 ml. of 4% potassium iodide solution, 2 ml. of 3% acetic acid, and 2 drops of starch solution as an indicator are added. The 0.1% *N*-bromosuccinimide solution—i.e., 1 ml. contains 1 mg. of *N*-bromosuccinimide—is run into the ascorbic acid solution drop by drop with continuous shaking after each addition until the end point is reached. From the number of milliliters of *N*-bromosuccinimide solution used in the titration process, the amount of ascorbic acid present can be readily calculated.

This method has been successfully applied to several kinds of injections obtainable in Egypt. One example is sufficient to illustrate the validity of the assay method. The injection consisted of 0.5 gram of ascorbic acid and 1 gram of calcium gluconate made up to 10 ml. with distilled water. One milliliter of this injection contained 50 mg. of ascorbic acid. One milliliter of this preparation was diluted with distilled water up to 50 ml. in a standard flask. Therefore, 5 ml. of this diluted solution should contain 5 mg. of ascorbic acid (see Tables VI and VII).

TABLETS. Ten tablets are weighed and powdered and an accurately weighed quantity of the powder, equivalent to 50 mg. of ascorbic acid, is introduced into a 50-ml. standard flask. Successive small quantities of distilled water (10 ml.) and glacial acetic acid (5 ml.) are added with continuous and vigorous shaking and the volume is made up to the mark with distilled water. The mixture is shaken well for 15 minutes and then filtered. The assay is carried out using 5 ml. of the filtrate, as described for injections. The result obtained, multiplied by 10, gives the amount of ascorbic acid in the original weight taken (see Tables VI and VII).

The assay can be carried out even on one tablet with reproducible results (see Table VIII).

Biological Fluids. DETERMINATION OF ASCORBIC ACID IN WHOLE BLOOD AND PLASMA. Five milliliters of oxalated whole blood or plasma are added, dropwise with shaking, to 5 ml. of 20% trichloroacetic acid in a 25-ml. centrifuge tube, and stirred to obtain a fine suspension. The suspension is allowed to stand 5 minutes, then centrifuged. The clear supernatant solution is filtered and 5 ml. of the filtrate are titrated immediately against 0.001% solution of *N*-bromosuccinimide; 1 ml. contains 0.01 mg. of *N*-bromosuccinimide (Table IX).

Table V. Comparison of Results by *N*-Bromosuccinimide and 2,6-Dichlorophenolindophenol

Dilution	Ascorbic Acid Soln., Ml.	Content, γ	Found by NBS, γ	Error, %	Found by Dye, γ	Error, %
10×, 100 γ per ml.	5	500	497	0.6	496	0.8
	2.5	250	248	0.8	248	0.8
	1	100	100	...	99	1.0
100×, 10 γ per ml.	5	50	50	...	50	...
	2.5	25	25	...	24.6	1.6
	1	10	10	...	10.5	5.0

Table VI. Estimation of Ascorbic Acid in Injections and Tablets by 0.1% *N*-Bromosuccinimide Solution

Sample	Diluted Ascorbic Acid Solution, Ml.	Ascorbic Acid Content, Mg.	0.1% NBS Used, Ml.	Ascorbic Acid Found, Mg.	Error, %
Injection	5	5	5.05	4.99	0.2
Tablet	5	5	5.1	5.04	0.8

Table VII. Comparison of Results by *N*-Bromosuccinimide and 2,6-Dichlorophenolindophenol

Sample	Ascorbic Acid per Ampoule or Tablet, Mg.	Found by NBS, Mg.	Error, %	Found by Dye, Mg.	Error, %
Injection, 10 ml.	500	494	1.2	495	1.0
Tablet	50	50.9	1.8	51	2.0
	500	499.9	0.02	503	0.6

Table VIII. Assay on Ten Tablets and One Tablet

No. of Tablets	Original Weight, Grams	Weight of Powder Assayed, Gram	Ascorbic Acid Content, Mg.	Ascorbic Acid Found, Mg.	Error, %
10	2.0	0.20	50	50.03	0.06
1	0.198	0.198	50	49.95	0.1
10	5.00	0.50	100	100.1	0.1
1	0.503	0.503	100	100.2	0.2

Table IX. Estimation of Ascorbic Acid in Blood and Urine by *N*-Bromosuccinimide and 2,6-Dichlorophenolindophenol

Blood Filtrate, Ml.	0.001% NBS Used, Ml.	Found, Mg. %	Dye Used, Ml. (1 Ml. = 0.117 Mg.)	Found, Mg. %
5	2.25	0.89	...	...
5	2.50	0.99	...	...
5	5.00	1.98	0.45	2.11
5	3.00	1.18	0.25	1.17
Acidulated Urine, Ml.	**0.01% NBS Used, Ml.**	**Found, Mg. %**	**Dye Used, Ml. (1 Ml. = 0.125 Mg.)**	**Found, Mg. %**
2	3.80	23.48	3.00	23.43
2	3.70	22.86	2.90	22.65
2	1.80	11.12	1.40	10.94
2	2.60	16.06	2.10	16.40
2	2.28	14.08	1.80	14.06
5	1.35	3.33	1.08	3.37
5	2.80	6.92	2.20	6.88

Determination of Ascorbic Acid in Urine. To 40 ml. of the urine sample (better freshly passed) 10 ml. of 20% metaphosphoric acid are added to retard the oxidation of ascorbic acid, and mixed well. Two or 5 ml. of the acidulated sample are measured accurately according to the ascorbic acid content; then 5 ml. of 4% potassium iodide solution and 2 drops of starch solution are added and titrated against 0.01% *N*-bromosuccinimide solution; 1 ml. contains 0.1 mg. of *N*-bromosuccinimide (Table IX).

Table X. Recovery of Added Ascorbic Acid to Whole Blood and Urine

Original Sample Content by NBS, Mg. %	Ascorbic Acid Added, Mg. %	Recovery of Ascorbic Acid Added by NBS, Mg. %	Recovery of Ascorbic Acid by Dye, Mg. %
1.06	1.00	0.99	1.04
Blood			
1.06	2.00	2.00	2.11
0.93	10.00	9.98	...
14.06	10.00	9.98	10.89
Urine			
14.06	20.00	19.97	20.74
22.75	35.00	35.04	35.06
3.35	2.00	1.96	2.02
14.07	14.00	14.04	14.05

Recovery of Ascorbic Acid Added to Whole Blood and Urine. Some recovery experiments were carried out in which the ascorbic acid added to whole blood and urine was in the same range, less than the amount originally present and also several times as great in amount as the amount originally present. The original sample content was estimated by means of *N*-bromosuccinimide; and the recovered ascorbic acid determined by *N*-bromosuccinimide and dye methods was expressed in terms of the added ascorbic acid (Table X).

Edible Fruits. The new method has been applied to the estimation of ascorbic acid in certain edible Egyptian fruits known to contain ascorbic acid—e.g., oranges, lemons, tomatoes, grapes, watermelons, and water cress. The fruits used in the estimation of ascorbic acid were almost ripe. The sampling and extraction of the material under examination must be carried out with the minimum delay, so that no significant change in ascorbic acid content takes place prior to analysis.

Each sample is squeezed or bruised and the juice is directly received in a known volume of 20% trichloroacetic acid, so that the juice is diluted with the stabilizing acid at least twice, to avoid the oxidation of ascorbic acid and also to precipitate interfering substances such as protein and thereby facilitate subsequent clarification of the extract. The acidulated juice is filtered and the determination is carried out on a known volume of the clear filtrate as described previously. The *N*-bromosuccinimide solution used in the titration is 0.01%—i.e., 1 ml. contains 0.1 mg. of *N*-bromosuccinimide (see Tables XI and XII).

RESULTS

Interfering Substances. The following substances which might interfere have no influence on the titration process: carbohydrates such as glucose, lactose, sucrose, and starch; diketogulonic acid; reductones and reductic acid; urea; uric acid and creatinine; alcohols; formaldehyde; acetone; ethyl acetate and acetoacetic ester; thiamine hydrochloride and riboflavine; oxalates, tartrates, and citrates; amino acids such as glycine, alanine, valine, and isoleucine; and ferrous and ferric salts. The only interfering substances that are also oxidized by *N*-bromosuccinimide before iodine is liberated from potassium iodide in acetic acid medium include sodium sulfite, sulfide, thiosulfate, and thiourea.

Experimental Error. From the results given in Tables IV to VII, it has been deduced that the error of the proposed method does not exceed ±2%. Every result is the average of at least duplicate or triplicate titration processes.

DISCUSSION

N-Bromosuccinimide acts as an oxidizing agent—e.g., it converts primary and secondary alcohols into the corresponding aldehydes and ketones, respectively (*2*). Its oxidizing action is peculiar and highly selective in many cases, as is shown in the oxidation of the 7α-hydroxyl group of cholic acid (*7*) and the 6β-hydroxyl group of cholestane-3β,5α,6β-triol by use of *N*-bromosuccinimide. Selective oxidation of a 3-acyl derivative of methyl cholate (*8*) to the 7-ketone can be accomplished in high yield with *N*-bromosuccinimide.

There seem to be no references in the literature to the reaction between *N*-bromosuccinimide and ascorbic acid. *N*-Bromosuccinimide in aqueous medium readily oxidizes an aqueous solution of ascorbic acid to dehydroascorbic acid, while *N*-bromosuccinimide is irreversibly reduced to succinimide with the formation of hydrogen bromide. Dehydroascorbic acid has been isolated in the form of its osazone. Succinimide has been separated from the reaction mixture and the formation of hydrogen bromide has

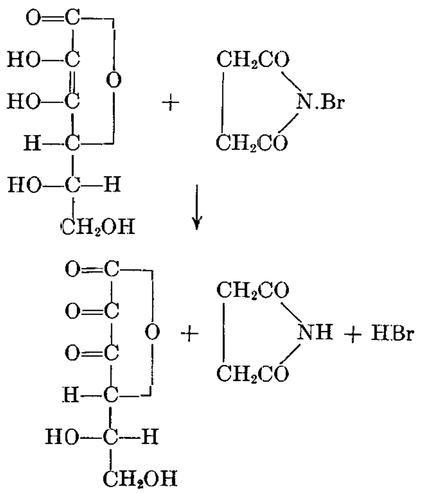
Table XI. Estimation of Ascorbic Acid in Fruits by *N*-Bromosuccinimide

Fruit Sample	Ascorbic Acid Determined by NBS, Mg. %
Orange (Balady)	72.78
Orange (Soccary)	56.15
Orange (Abo-Sorrah)	52.57
Orange (Shammouti)	35.59
Mandarin	37.57
Lemon Banzaheir	32.00
Tomato	24.80
Grapefruit	29.84
Watermelon	27.90
Water cress	13.02
Pomegranate	33.59

Table XII. Comparative Determination of Ascorbic Acid in Fruits

Fruit Sample	Found by NBS, Mg. %	Found by Dye, Mg. %
Orange (Seifi)	39.55	39.60
Orange (Seifi)	35.63	36.00
Water cress	17.79	18.40
Watermelon	27.68	28.23
Lemon Banzaheir	45.44	46.56
Tomato	27.66	29.10
Guava	43.96	44.62
Grapefruit	24.90	25.29
Mango	55.72	57.04

been confirmed. The reaction proceeds quantitatively in equimolecular concentrations according to the equation:



The fact that ascorbic acid reacts very rapidly with *N*-bromosuccinimide, whereas many of the interfering substances react more slowly or even do not react at all, provides a reliable titrimetric method for the determination of ascorbic acid.

N-Bromosuccinimide is an oxidizing agent and thus can liberate iodine from potassium iodide in aqueous acetic acid medium, but it oxidizes ascorbic acid to dehydroascorbic acid preferentially. Until all the ascorbic acid present in the solution is oxidized, no iodine is liberated from potassium iodide. The slightest excess of *N*-bromosuccinimide added, after all the ascorbic acid content has been oxidized, will liberate iodine from potassium iodide, which is easily detected by the blue color developed with a few drops of starch solution added at the beginning of the titration process. The end point is thus easily observed by the appearance of a blue color in the ascorbic acid solution or extract.

The presence of other reducing substances does not interfere with the titration with *N*-bromosuccinimide, since iodine is selectively liberated from potassium iodide before reducing substances present, other than ascorbic acid, are oxidized by *N*-bromosuccinimide, and consequently the end point is easily determined in the presence of starch. The end point is definitely blue in the case of pure solutions and pharmaceuticals, but violet in the case of biologicals and fruits.

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Received for review February 3, 1954. Accepted November 22, 1954.

Method of Assay for Ethylenimine Derivatives

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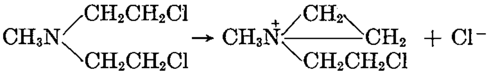
A method was devised for the determination of ethylenimine derivatives, which are being studied experimentally for use in the treatment of cancer. It is based on a rapid reaction between the ethylenimino group and thiosulfate ion at pH 4. This reaction consumes 1 mole of acid for each ethylenimino group. A large excess of thiosulfate is needed to suppress competing reactions. As this excess is difficult to measure with precision, the acid consumption is measured instead, by adding standard acid and thiosulfate and titrating the excess acid with standard alkali. The method has been studied for the assay of the following ethylenimine derivatives and homologs: 2,4,6-tris(1-aziridyl)-*s*-triazine (triethylenemelamine), 2-amino-4,6-bis(1-aziridyl)-*s*-triazine (diethylenemelamine), *N,N′,N″*-triethylenephosphoramide, *N,N′,N″*-triethylenethiophosphoramide, *N*-(3-oxapentamethylene)-*N′,N″*-diethylenephosphoramide, *N,N′,N″*-tris(1-methylethylene) phosphoramide, *N,N′,N″*-tris(1,1-dimethylethylene) phosphoramide, *p*-toluenesulfon-*N*-ethyleneamide, and *N,N′*-diethylenebenzene-1,3-disulfonamide. The standard deviation of these determinations, where applicable, is in the neighborhood of ±0.1%.

SEVERAL derivatives of ethylenimine have recently been studied as possible agents for the treatment of certain types of cancer. These compounds have physiological effects similar to those of the nitrogen mustards, which have been used for the palliative treatment of cancer (*3*). However, the ethylenimines are reported to be easier to administer and to bring about unpleasant side effects less often (*2*).

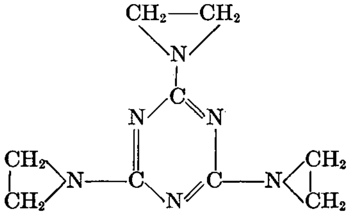
When a nitrogen mustard is dissolved in water, it is transformed to an ethylenimonium ion (*1*). Equation 1 shows such a reaction for methylbis(β-chloroethyl)amine, a typical nitrogen mustard:



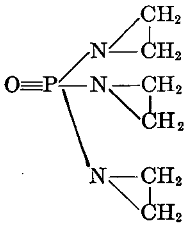
(1)

The ethylenimonium ion is thought to be the active chemotherapeutic agent.

The ethylenimino group has a structure similar to that of the ethylenimonium ion, except that the nitrogen atom does not have a positive charge. 2,4,6-Tris(1-arizidyl)-*s*-triazine (triethylenemelamine) (I), also known as TEM, is the ethylenimine derivative which has been most thoroughly studied clinically. Another typical ethylenimine derivative is *N,N′, N″*-triethylenephosphoramide (II).



I

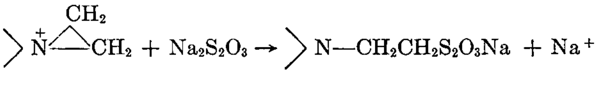


II

A method for the assay of the ethylenimines was needed both for the regulation of dosages and for synthetic research.

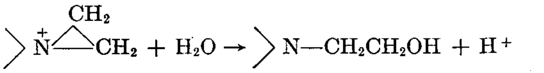
THEORY OF METHOD

Golumbic, Fruton, and Bergmann (*1*) have shown that the ethylenimonium ion can be determined in aqueous solution by reaction with thiosulfate according to Equation 2.



$$>\overset{+}{N}\overset{CH_2}{\triangle}CH_2 + Na_2S_2O_3 \rightarrow\ >N-CH_2CH_2S_2O_3Na + Na^+ \qquad (2)$$

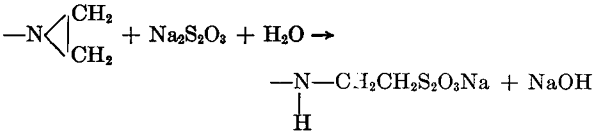
Since each molecule of methylbis (β-chloroethyl)amine has two β-chloroethyl groups, it reacts with 2 molecules of thiosulfate. The titration is conducted by treating the solution of the nitrogen mustard with excess standard thiosulfate solution and then back-titrating with standard iodine solution. The data presented by these authors show that the results are not quantitative. The reason is probably the occurrence of side reactions mentioned by these authors. For example, the imonium ion can undergo hydrolysis as shown in Equation 3.



$$>\overset{+}{N}\overset{CH_2}{\triangle}CH_2 + H_2O \rightarrow\ >N-CH_2CH_2OH + H^+ \qquad (3)$$

Another possible side reaction is dimerization, which produces a piperazine ring.

Preliminary work showed that low results were also obtained when the thiosulfate reaction was applied to triethylenemelamine, but the reaction could be speeded by lowering the pH. Furthermore, when the reaction was conducted at pH 4 (found to be the optimum pH), about 4 equivalents of acid per mole of triethylenemelamine were consumed. A partial explanation of the acid consumption may be the reaction of Equation 4. This would account for 3 equivalents of acid per mole of triethylenemelamine. Monobasicity of the reaction product would account for the additional equivalent.



$$-N\begin{matrix}CH_2\\ |\\ CH_2\end{matrix} + Na_2S_2O_3 + H_2O \rightarrow \underset{H}{\underset{|}{-N}}-CH_2CH_2S_2O_3Na + NaOH \qquad (4)$$

The basicity of the reaction product was verified by running a potentiometric titration curve on the reaction mixture. This curve showed two breaks, at pH 4 and 8. The difference between the breaks corresponded to 1 equivalent of acid per mole of triethylenemelamine. The phosphoramides so far investigated give reaction products with thiosulfate which are not quite so basic, and consume less than 1 equivalent of acid per mole between pH 4 and 8.

The method finally chosen is to use a large excess of thiosulfate solution in high concentration. This suppresses side reactions. The thiosulfate consumption is not measured because of the large excess involved, but the acid consumption is measured instead. The effect of the basicity of the reaction product is cancelled by back-titrating the solution to the phenolphthalein end point after the reaction has proceeded at pH 4 for 0.5 hour.

A similar method in which the titration is made directly with acid to the phenolphthalein end point, in a refluxing acetone-water solution, is used by Ross (*4*).

DESCRIPTION OF METHOD

To an accurately weighed portion of about 3 meq. of sample (1 equivalent corresponds to 1 ethylenimino group) add 50 ml. of sodium thiosulfate solution (20 grams of $Na_2S_2O_3.5H_2O$ per 100 ml.), followed by 1 drop of methyl orange indicator solution. Titrate the solution immediately with 0.1*N* hydrochloric acid to a pH of 4, as indicated by the methyl orange color. (The proper color may be judged by comparison with a pH 4 buffer solution containing methyl orange.) If the end point does not persist for at least 10 seconds, continue the titration until it does. Allow the solution to stand for 0.5 hour. Add several drops of phenolphthalein indicator solution and titrate with 0.1*N* sodium hydroxide to the phenolphthalein end point. Run a blank in the same manner on 50 ml. of the sodium thiosulfate solution without the sample. The acid consumption minus the consumption of alkali, corrected for the blank, is equivalent to the ethylenimino groups present.

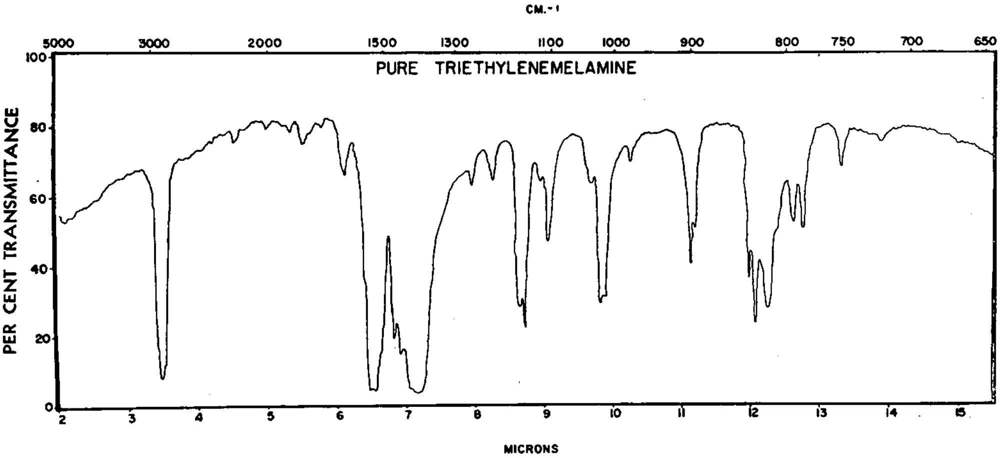


Figure 1. Spectrum of pure triethylenemelamine

DISCUSSION OF RESULTS

Assay of Triethylenemelamine. Table I shows assay values of several samples of triethylenemelamine. The standard deviation, in the neighborhood of ±0.2%, indicates that the method has adequate precision for the purpose for which it is intended. In general, the samples which are known to be crude or which have been aged give lower values, as is to be expected. However, the aged samples

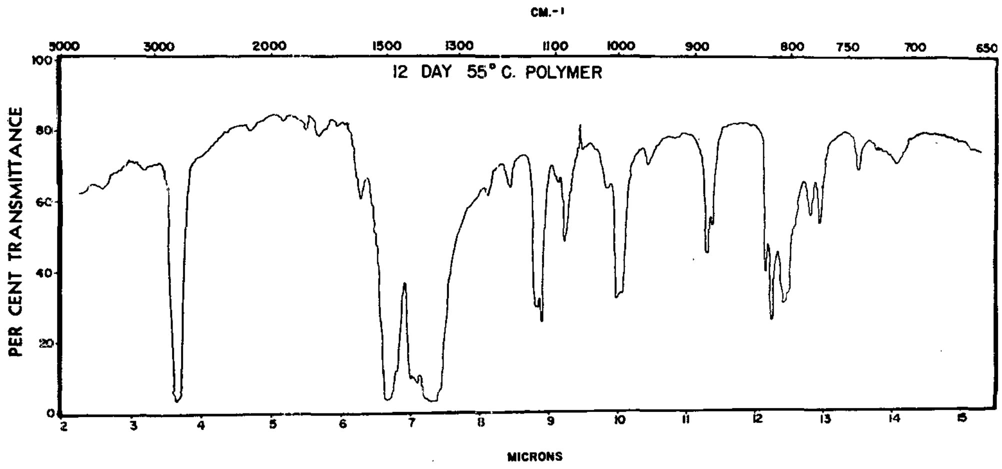


Figure 2. Spectrum of polymer prepared by heating triethylenemelamine in the dry state at 55° C. for 12 days

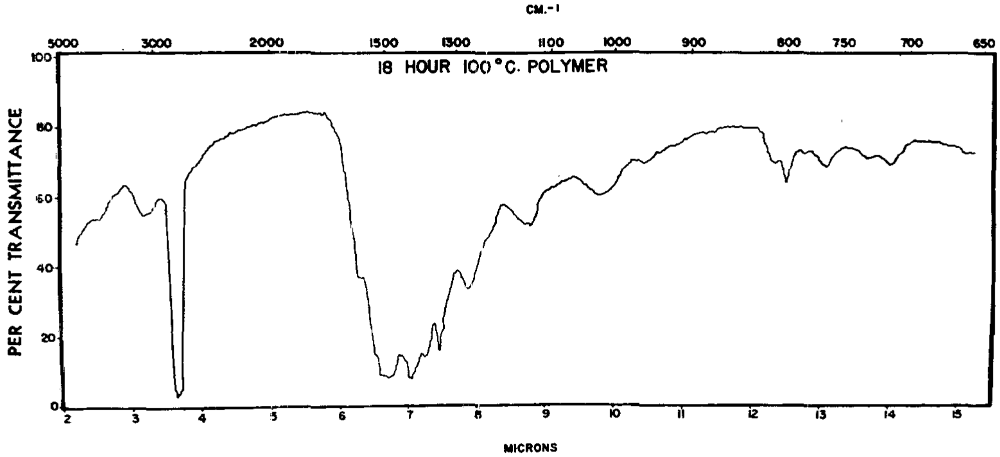


Figure 3. Spectrum of polymer prepared by heating triethylenemelamine in the dry state at 100° C. for 18 hours

Table I. Assay of Triethylenemelamine Samples

Description	Values, %
Purified	99.7, 99.6, 99.6 99.7, 99.8, 99.8
Crude 1	80.9, 82.5
Crude 2	95.5, 95.5
Recrystallized	98.2, 98.2
Twice recrystallized	98.9, 98.9
Two years old	97.3, 97.1, 97.4
One year old	98.9, 99.4
One year old	99.7, 99.7
Purified (slightly turbid in water)	99.8, 99.7
Purified (decomposition point slightly low)	99.5, 99.5

were not aged under ideal conditions, as no effort was made to keep them absolutely dry.

Assay of Triethylenemelamine Polymers. Triethylenemelamine shows a tendency to polymerize. Samples of triethylenemelamine which have been aged always show the presence of polymer by forming a milky solution when dissolved in water. Polymerization of triethylenemelamine can be induced by heating the sample, either with water or in the dry state. Table II shows assay values for some of the triethylenemelamine polymers. The first polymer, which was simply isolated from the crude sample by filtration, has a low assay value. The second polymer, prepared by heating triethylenemelamine with water, likewise shows low purity. The three last polymers in the table were prepared by heating triethylenemelamine in the dry state. The table shows that heating at 55° C. for up to 12 days produced only a minor effect in the assay value. It is possible that these samples are only partially polymerized. They contain appreciable insoluble matter, but a quantitative determination of solubility was not made and the amount of insoluble may be small. However, the sample which was heated for 18 hours at 100° C. gave a low assay value. Polymerization in this case was probably fairly complete.

Independent evidence for the relation between the polymers and the pure compound was obtained from infrared spectra. Figures 1, 2, and 3 show spectra of pure triethylenemelamine and the two polymers prepared by heating triethylenemelamine in the dry state at 55° C. for 12 days and at 100° C. for 18 hours. The spectra of the pure material and of the 55° C. polymer are very similar, whereas that of the 100° C. polymer is different.

Assay of Other Ethylenimines. Table III shows assay values obtained on other ethylenimines and ethylenimine homologs. The homolog with 1 methyl group on each ethylenimine ring yields almost quantitative values, whereas the homolog with 2 methyl groups on each ethylenimine ring is only partially titrated. Steric hindrance may be responsible. The procedure is not applicable to the two sulfonamide derivatives shown in the table.

Stability Studies on Ethylenimines. When clinical trials were started on the ethylenimines, it was important to know in which form the compound would be most stable—in the solid

Table II. Assay of Triethylenemelamine Polymers

Description	Values, %
Isolated from crude sample	3.1, 3.0
TEM	
Heated with water on steam bath 1 hour	0.7, 0.6
Heated 24 hours at 55° C.	97.5
Heated 18 hours at 100° C.	0.2
Heated 12 days at 55° C.	91.3

Table III. Assay of Other Ethylenimines and Homologs

Designation	Values, %
2-Amino-4,6-bis(1-aziridyl)-s-triazine (diethylenemelamine)	99.7, 99.7
N,N',N''-Triethylenephosphoramide[a]	99.6, 99.3
N,N',N''-Triethylenethiophosphoramide[a]	99.9, 100.0
N-(3-Oxapentamethylene)-*N',N''*-diethylenephosphoramide	99.3
N,N',N''-Tris(1-methylethylene)phosphoramide	98.6, 98.4
N,N',N''-Tris(1,1-dimethylethylene)phosphoramide	53.8, 52.6
p-Toluenesulfon-*N*-ethyleneamide	[b]
N,N'-Diethylenebenzene-1,3-disulfonamide	[b]

[a] Weighings conducted under stream of dried nitrogen because of hygroscopicity of compound.
[b] Reaction too slow to be practical.

state, or dissolved in physiological saline solution, phosphate buffer solution, or sesame oil. Accordingly, stability studies in these solvents and in the dry state were carried out on *N,N′,N″*-triethylenephosphoramide and *N,N′,N″*-triethylenethiophosphoramide. The analytical procedure for these determinations, except for the solutions in sesame oil, was the same as the procedure already described. (With the phosphate buffer solution it was necessary to run careful blanks to allow for the effect of the buffer in consuming alkali.) For the solution of the thiophosphoramide in sesame oil a modification was used in which each addition of the acid or alkali was accompanied by vigorous shaking. The purpose was to extract the compound from the sesame oil layer into the water layer. But with phosphoramide dissolved in sesame oil, this extraction into the aqueous phase was much too slow. Therefore, boiling thiosulfate solution was added to the sample, followed immediately by a measured volume of boiling standard hydrochloric acid solution. The titration was then completed at the boiling point with standard hydrochloric acid and sodium hydroxide solutions. The procedure gave results of relatively poor precision, but served to indicate the stability in a satisfactory manner. Results of these determinations show that the aqueous solutions, both physiological saline and phosphate buffer, were unstable. However, the compound in the solid state and in the sesame oil solution showed little, if any, loss in strength over the period studied.

ACKNOWLEDGMENT

The authors are greatly indebted to Frederick S. Philips, Sloan-Kettering Institute for Cancer Research, New York, N. Y., who suggested at the outset of the study that the thiosulfate titration method used for the nitrogen mustards (*1*) might be applied to the assay of the ethylenimines. Philips also obtained some preliminary data which indicated that the reaction of triethylenemelamine with thiosulfate was accompanied by a release of acid. The authors also wish to thank Erwin Kuh and Doris Seeger for furnishing the samples of the phosphoramides, P. F. Dreisbach and C. M. Hofmann for furnishing the other samples, D. N. Kendall for the infrared work, and Z. F. Smith for obtaining some of the results in the stability studies.

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RECEIVED for review June 17, 1954. Accepted December 1, 1954. Presented at the Meeting-in-Miniature of the North Jersey Section, AMERICAN CHEMICAL SOCIETY, Newark, N. J., January 26, 1953.

Volumetric Determination of Nitrocellulose and Nitroguanidine

By Transnitration of Salicylic Acid

HARRY STALCUP and RICHARD W. WILLIAMS
U. S. Naval Powder Factory, Indian Head, Md.

This investigation was undertaken because of the need for a direct chemical method for the determination of per cent nitrogen in nitrocellulose for specialized applications where the nitrometer was either inapplicable or inaccurate. Nitrogen in nitrocellulose samples of various levels of nitration and nitramine nitrogen in nitroguanidine were determined volumetrically by the transnitration of salicylic acid in sulfuric acid solution, the sample being used as the nitrating agent, followed by reduction of the resulting nitrosalicylic acid with standard titanous chloride. Determinations on samples of known nitrogen content showed a standard deviation of 0.02% and were in good agreement with nitrometer values. The method offers a new approach to the determination of nitramine-type ingredients in propellant-powder formulations.

THE determination of nitrate nitrogen in nitrocellulose and nitramine nitrogen in nitroguanidine is of importance in the production of gun and propellant powders. A nitrometer is ordinarily used, but because of their low solubility in cold sulfuric acid, some nitrocellulose fractions prepared in this laboratory under various experimental conditions failed to yield their true nitrogen values in the nitrometer. For this reason a rapid and accurate titration procedure was sought. A procedure has been developed, based in part on the Moore (*4*) modification of the Förster (*2*) salicylsulfonic acid method for nitrate nitrogen, in which the nitrate compound is used as the agent for the nitration of salicylic acid in a sulfuric acid medium. The resulting nitro compound is then reduced with standard titanous chloride by the Knecht-Hibbert method (*3*). Compounds containing both nitrate and nitramine nitrogen—such as β-nitroxyethylnitroguanidine—have been analyzed, and nitroguanidine in gun propellant compositions has been determined. The compound produced in the salicylic acid–nitrate reaction has been identified as 5-nitrosalicylic acid.

APPARATUS

Cylinder of carbon dioxide or nitrogen gas.

Heat source, Variac-controlled hot plates or heating mantle to fit 250-ml. refluxing flask.

Refluxing flask, 250 ml.

Soxhlet unit, 20-ml. capacity siphoning cup.

Reflux condenser, water-cooled to fit Soxhlet unit.

Reflux condenser, water-cooled with ground-glass standard-taper tip.

Glass bottle of 2-liter capacity, equipped with an automatic buret for storage and use of the titanous chloride solution. The siphon tube and buret must be connected in such a way that only carbon dioxide or nitrogen gas, supplied from a tank, will be drawn into the reagent bottle as the solution is used. The bottle should be covered with black paint or black paper to exclude light.

Refluxing flask, 500-ml. capacity, with a round bottom and two necks. One of the necks should be of ground glass with a standard taper to fit the reflux condenser.

REAGENTS

TITANOUS CHLORIDE SOLUTION (0.3*N*). For each liter of titanous chloride solution, 225 ml. of 20% titanium trichloride is mixed with 100 ml. of 38% hydrochloric acid. The mixing operation should take place before diluting, and in these operations the solution should be protected from the air as much as possible by means of carbon dioxide. The solution should then be thoroughly mixed by means of a current of carbon dioxide and stored in reagent bottle. An alternative procedure for making titanous chloride from titanous hydride is as follows:

Table I. Per Cent Nitrogen Content of Various Compounds

Comparison of Salicylic Acid Nitration Method with Nitrometer

Compound	Nitrometer	Salicylic Acid–Titanous Chloride Method Test No. 1	2	3	4	5	Av.	Calcd.	Range	S.D.
Nitrocellulose	11.73	11.74	11.77	11.74	11.75	11.75	11.75	...	0.04	0.02
Nitrocellulose	12.60	12.61	12.61	12.60	12.62	12.61	12.61	...	0.02	0.01
Nitrocellulose	13.15	13.17	13.16	13.16	13.14	13.15	13.16	...	0.03	0.01
Nitroguanidine	13.41	13.39	13.40	13.39	13.41	13.40	13.40	13.46	0.02	0.01
β-Nitroxyethylnitroguanidine	...	14.56	14.51	...	...	...	14.54	14.50	...	...
Ammonium nitrate	...	17.44	17.46	...	...	...	17.45	17.50	...	...

Table II. Per Cent Nitroguanidine in Nitroguanidine Propellant

Replica	Salicylic Acid Nitration–Titanous Chloride Reduction Uncorrected	Corrected[a]	Water Extraction–Gravimetric Method
1	58.78	58.95	59.04
2	58.70	58.87	58.88
3	58.83	59.00	59.04
4	58.85	59.02	58.65
5	58.89	59.06	58.98
Av.	58.81	58.98	58.92
Range	0.19	0.19	0.39
S.D.	0.07	0.07	0.16

[a] Corrected for nitration of 13.41% (99.63% theoretical); obtained by dividing uncorrected values by 99.63.

To 22 grams of titanous hydride weighed into a 1-liter Erlenmeyer flask, 200 ml. of concentrated hydrochloric acid is added slowly. The mixture is heated on a steam bath (caution, hydrogen evolved) in a hood in an atmosphere of carbon dioxide. After the reaction has subsided, 200 ml. of hydrochloric acid is added and the solution allowed to stand for 1 hour. The solution is then filtered (under carbon dioxide), diluted to 2 liters, mixed, and stored as previously described.

For standardizing titanous chloride solution the following procedure is recommended. A current of carbon dioxide is passed for 5 minutes through the 500-ml. refluxing flask equipped with an inlet tube. From a buret 25 ml. of 0.2*N* solution of potassium dichromate (reagent grade) is added, followed by 50 ml. of 10% sulfuric acid solution. The titanous chloride solution is used for the titration, 3 drops of a 0.5% aqueous solution of sodium diphenylbenzidine sulfonate being added near the end point. The indicator color change is from a distinct light blue to bright green. Diphenylamine (0.5%) in strong sulfuric acid may be used instead of sodium diphenylbenzidine. The color change at the end point is from blue to sea green.

Ferric Ammonium Sulfate Solution (0.15*N*). For each liter of ferric ammonium sulfate solution 75 grams of hydrated ferric ammonium sulfate [$Fe_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$] is mixed with 25 ml. of 95% sulfuric acid. The mixing should be done by means of a current of carbon dioxide. The solution is standardized as follows: The air in a two-necked refluxing flask is displaced with carbon dioxide. A solution of 40 to 45 ml. of ferric ammonium sulfate is accurately measured into the flask and 25 ml. of 1 to 1 hydrochloric acid solution and 50 ml. of water are added. The solution is titrated with the 0.3*N* titanous chloride until near the end point; then 5 ml. of 20% ammonium thiocyanate solution is added. The titration is continued until the red color just disappears. Temperature and buret corrections are applied to the observed readings, and the normality of the ferric ammonium sulfate solution is calculated.

Ammonium Thiocyanate (20%). Twenty grams of ammonium thiocyanate (c.p.) is dissolved in 100 ml. of distilled water.

Acetic Acid (70%). Seven hundred milliliters of glacial acetic acid is mixed with 300 ml. of distilled water.

Sulfuric-Salicylic-Acetic Acid Mixture. One part by volume of glacial acetic acid is mixed with 9 parts by volume of concentrated sulfuric acid; then 100 grams of dry salicylic acid is dissolved in 1000 ml. of the mixture.

Sulfuric-Salicylic Acid Mixture. Twelve grams of dry salicylic acid is dissolved in 1000 ml. of concentrated sulfuric acid.

Dry Carbon Tetrachloride.

PROCEDURE

Determination of Nitrogen in Nitroguanidine or Nitrocellulose. A 0.30 ± 0.05-gram sample is weighed accurately into a glass weighing bottle of 20- to 25-ml. capacity. Ten milliliters of the sulfuric-salicylic-acetic acid mixture is added. The solution is then stirred carefully until dissolved and allowed to stand for about 20 minutes.

Nitrocellulose samples above 13% in nitrogen content must first be dissolved in 10 ml. of concentrated sulfuric (room temperature) and left in contact with the acid for a period no longer than 20 minutes. Then 10 ml. of the sulfuric-salicylic-acetic acid solution is added with stirring and the mixture is allowed to stand for an additional 10 minutes.

The contents of the weighing bottle are transferred to a 500-ml. two-necked refluxing flask attached to a carbon dioxide tank and washed in with 40 to 50 ml. of distilled water. After the air in the flask has been replaced with carbon dioxide, 75 ml. of standard 0.3*N* titanous chloride solution is added. A 20 to 25% excess of reagent is sufficient. The flask is attached to a water-cooled reflux condenser and the contents are boiled for 1 minute. Then the heat is removed and while the flask is still attached to the condenser, it is cooled to room temperature by means of a container of cool running water. It may be necessary to increase the flow of carbon dioxide before cooling to prevent air from entering the reaction chamber and oxidizing the titanous chloride. The flask is removed from the condenser, 1 ml. of a saturated solution of ammonium thiocyanate indicator is added, and the excess titanous chloride is titrated with 0.15*N* ferric ammonium sulfate solution to the red end point. A blank is run on the reagents, only the sample being omitted, and any significant correction applied.

Calculations.

$$\frac{\text{Ml. of TiCl}_3\text{ used} \times N\text{ of TiCl}_3 \times 0.002335 \times 100}{\text{Grams of sample}} = \text{per cent of nitramine or nitrate nitrogen}$$

where 0.00235 = gram of nitrogen equivalent to 1 ml. of normal titanous chloride

$$\frac{\text{Ml. of TiCl}_3\text{ used} \times N\text{ of TiCl}_3 \times 0.01735 \times 100}{\text{Grams of sample}} = \text{per cent nitroguanidine}$$

where 0.01735 = gram of nitroguanidine equivalent to 1 ml. of normal titanous chloride.

Smaller samples of nitroguanidine may be used with correspondingly smaller volumes of titanous chloride.

Volumetric Procedure for Determination of Nitroguanidine in Propellant Compositions. A 0.5-gram sample (ground in a Wiley mill to 20-mesh particle size) is weighed accurately into a small paper extraction thimble. A plug of glass wool is inserted into the thimble and the latter is dropped into a small Soxhlet extraction tube having a siphoning-cup capacity of approximately 20 ml. The sample is extracted for 3 hours with dry carbon tetrachloride under a water-cooled condenser. A heating mantle or other Variac-controlled heat source is adjusted so that the solvent vapors will condense in an almost continuous stream above the sample.

After extraction the paper thimble is removed and both the thimble and Soxhlet tube are dried thoroughly. The thimble is then reinserted into the Soxhlet tube. A 250-ml. refluxing flask containing 40 ml. of 70% acetic acid is attached to the Soxhlet tube and the sample extracted for 2.5 hours. The heat source should be adjusted so that a siphoning cycle is obtained in 6 minutes or less. After the extraction the siphoning cup is allowed nearly to fill (15 to 20 ml.) and the heat is then turned off. The refluxing flask containing the nitroguanidine and lead acetate in 20 to 25 ml. of acetic acid is detached and cooled in an ice bath. A cold solution containing 85 ml. of the sulfuric-salicylic acid mixture is introduced into the flask and the contents are thoroughly mixed. The solution is permitted to stand 15 to 30 minutes, then it is diluted with 150 ml. of water, and transferred to a 500-ml. round-bottomed two-necked refluxing flask. Carbon dioxide is bubbled through the solution for 5 minutes and 75 ml. of 0.3*N* titanous chloride (or a calculated 25% excess) is added. The flask is attached to a water-cooled condenser, the carbon dioxide flow is continued, and the contents are boiled for 2 or 3 minutes. The heat source is removed and while the flask is still attached to the condenser, it is cooled to room temperature, by means of a vessel of cold running water. The flask is detached from the condenser, 5 ml. of a 20% solution of ammonium thiocyanate indicator is added, and the excess of titanous chloride titrated with 0.15*N* ferric ammonium sulfate to the red-brown end point. A blank is run on the reagents for the presence of reducible impurities, and any significant corrections are applied.

Calculations.

$$\frac{\text{Ml. of TiCl}_3\text{ consumed} \times N\text{ of TiCl}_3 \times 0.01735 \times 100}{\text{Grams of sample}} = \text{per cent nitroguanidine}$$

DISCUSSION

The described method was originally designed for "specialty" applications—i.e., for samples of nitrocellulose either too small or too insoluble in cold sulfuric acid to be readily determined by the nitrometer. However, standard lots of nitrocellulose and nitroguanidine were used in comparing the accuracy and precision of the new method with the nitrometer.

The results given in Table I on three lots of nitrocellulose and one lot of nitroguanidine show a maximum range of 0.04% and a standard deviation no greater than 0.02% in any case. Excellent agreement with the nitrometer was also obtained. Duplicate determinations on ammonium nitrate and β-nitroxyethylnitroguanidine indicate further applicability of the method.

A practical application of the method is demonstrated by the results shown in Table II on a typical nitroguanidine propellant. A range of 0.19% and a standard deviation of 0.07% indicate a more satisfactory degree of precision for powder analysis than that obtainable by a water extraction–gravimetric method.

Table III. Volumetric Determination of Nitrocellulose of 12.60% Nitration by Salicylic Acid–Nitration Method

Test No.	Refluxing[a] Time, Min.	Nitrocellulose, G.	N, %
1	5	0.1720	12.66
2	5	0.2655	12.69
3	5	0.3258	12.64
4	5	0.2184	12.71
5	5	0.2776	12.66
6	5	0.2810	12.68
7	3	0.2358	12.63
8	3	0.3296	12.64
9	2	0.2547	12.63
10	2	0.4099	12.61
11	1	0.3387	12.62
12	1	0.3043	12.61
13	1	0.3919	12.61

[a] Reflux time begins when first drop falls from condenser.

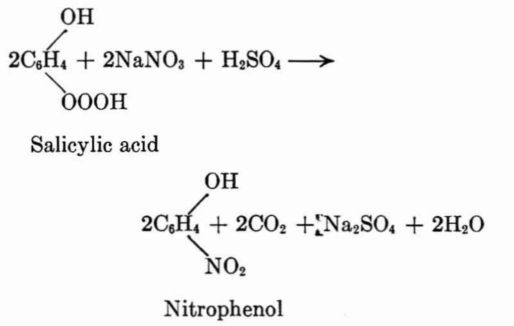
Commercial nitroguanidine used in powder manufacture usually ranges between 13.39 and 13.43% in nitration values as compared with the theoretical value of 13.46%. Therefore, for an accurate determination of the nitroguanidine content of a powder, the nitration value of the nitroguanidine should be known.

Table III shows experimental results obtained on a sample of nitrocellulose of 12.60% nitration. The refluxing period becomes a critical factor if continued beyond 2 minutes.

In Table IV, nitrocellulose of 13.15% nitration gave unreliable results when the procedure for the lower nitrations was used. However, when the sample was first dissolved in 10 ml. of pure sulfuric acid for a contact period of 20 to 30 minutes and then the sulfuric-salicylic-acetic acid reagent added for an additional contact time of 10 to 15 minutes, results agreed closely with the nitrometer values. No serious difficulty was encountered with the nitroguanidine determination. However, in order to make the procedure applicable to a determination of the nitroguanidine in a gun propellant, further investigation was necessary. Seventy per cent acetic acid has been found to be the strongest concentration permissible to ensure a minimum solubility of the nitrocellulose during the extraction of nitroguanidine and other compounds from the powder. During this investigation 85 ml. of concentrated sulfuric acid containing 1 gram of salicylic acid was found to be the minimum quantity that could be added to 25 ml. of 70% acetic acid containing nitroguanidine in solution and still effect quantitative nitration.

IDENTIFICATION OF NITRO COMPOUND

Conflicting statements appear in the literature regarding the identity of the nitro compound formed from the action of nitrates on salicylic acid in sulfuric acid medium. In his extensive work on the estimation of nitrates in fertilizers by the Förster method (*2*), Moore (*4*) suggested the following reaction:



$$2C_6H_4(OH)(COOH) + 2NaNO_3 + H_2SO_4 \longrightarrow 2C_6H_4(OH)(NO_2) + 2CO_2 + Na_2SO_4 + 2H_2O \qquad (1)$$

The nitro compound was reduced to the corresponding amine with sodium thiosulfate and the nitrogen then determined by the Kjeldahl method. Dickinson (*1*) stated that the salicylic acid is converted to 5-nitrosalicylic acid and submitted evidence to show that aminosalicylic acid is the derivative produced upon reduction of the nitro compound with sodium thiosulfate.

The identity of the nitro compound produced is only of academic interest because its final measurement, whether based on the modified Kjeldahl or the method described here, depends only on the presence of a nitro group. However, because of the

Table IV. Nitrogen in Nitrocellulose of 13.15% Nitration

(Determined with two methods)

	Salicylic Acid–Nitration Method				
	Original		Modified		
			Contact Time, Min.		
Test No.	Contact time in salicylic-sulfuric mixture, min.	N, %	Sulfuric acid	Sulfuric-salicylic-acetic mixture	N, %
1	10	12.24	15	10	13.17
2	10	12.03	15	15	13.16
3	15	13.17	20	10	13.14
4	15	13.14	20	10	13.16
5	15	12.87	20	15	13.15
6	15	13.16	20	15	13.16
7	15	13.01	20	20	13.21
8	20	13.26	15	25	13.26
9	20	13.41	30	15	13.16
10	20	13.40	60	15	12.82
11	30	13.62	90	10	12.87
12	35	13.82	120	20	12.47
13	35	13.86	...	..	...

Table V. Analysis of Synthesized Nitro Compound

	%			
Compound	Carbon	Hydrogen	NO_2 by $TiCl_3$ reduction	Salicylic acid (alkali titration)
Synthesized nitro compound, found	45.79	2.76	25.19	75.68
5-Nitrosalicylic acid, calcd.	45.91	2.75	25.12	75.43
3-Nitrosalicylic acid, calcd.	41.80	3.50	22.88	68.66
3-Nitrosalicylic acid (anhydrous), calcd.	45.91	2.75	25.12	75.43
o-Nitrophenol, calcd.	51.80	3.62	10.07	...

conflicting opinions expressed, an effort was made to identify the nitro compound produced.

Salicylic acid in sulfuric acid solution was added to an excess of sodium nitrate. The resulting solution was then poured over cracked ice and the insoluble nitro compound filtered off, washed, recrystallized twice, and dried at 125° C. No carbon dioxide generation was observed during the reaction, an indication that the carboxyl group was not displaced.

Nitrogen was determined by the titanous chloride reduction method of Knecht and Hibbert (*3*). Table V shows a comparison of the actual analysis of the synthesized nitro compound with the calculated analysis of ortho-nitrophenol and the 3-nitro and 5-nitro isomers of nitrosalicylic acid.

Melting points for these compounds are as follows:

Compound	Melting Points, ° C.
Synthesized nitro compound	185–192
5-Nitrosalicylic acid	228–230
3-Nitrosalicylic acid	123–125; 148–149 (anhydrous)
o-Nitrophenol	44–45

This comparison further substantiates the conclusion that the nitro compound formed is essentially 5-nitrosalicylic acid with a small amount of the 3-nitro isomer.

No attempt was made to identify the nitroguanidine–salicylic acid reaction products. However, conclusions based on the identity of the nitration products formed from the sodium nitrate–salicylic acid reaction in sulfuric acid indicate the following reactions:

$$H_2NC(=NH)NHNO_2 + C_6H_4(OH)COOH \xrightarrow[25°\ C.]{H_2SO_4}$$

Nitroguanidine Salicylic acid

$$O_2NC_6H_3(OH)COOH + [H_2NC(=NH)NH_2]_2 \cdot H_2SO_4 \qquad (2)$$

Nitrosalicylic acid Guanidine sulfate

$$O_2NC_6H_3(OH)COOH + 6TiCl_3 + 6HCl \longrightarrow$$
$$H_2NC_6H_3(OH)COOH + 6TiCl_4 + 2H_2O \qquad (3)$$

Aminosalicylic acid

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(1) Dickinson, W. E., ANAL. CHEM., **26**, 777 (1954).
(2) Förster, O., *Chem. Ztg.*, **13**, 229 (1889); **14**, 1674 (1890).
(3) Knecht, E., and Hibbert, E., "New Reduction Methods in Volumetric Analysis," Longmans, Green, and Co., New York, 1925.
(4) Moore, H. C., *J. Ind. Eng. Chem.*, **12**, 669 (1920).

RECEIVED for review October 1, 1954. Accepted December 10, 1954.

Analytical Distillation in Miniature Columns

Equipment and Operation

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Miniature distillation columns give excellent separations of 15- to 50-ml. samples equal to those obtainable with proportionally larger samples on macro high-efficiency laboratory columns. Controls and accessories adapted to the needs of the smaller columns are necessary to obtain such performance. Among these are a closely controlled power supply for the distilling flask heater, a receiver system that avoids mixing of fractions and allows accurate measurement of distillate volumes, and a vacuum system designed for leak-free operation. Spinning-band columns are especially useful for vacuum distillation.

THE need for fractional distillation of small quantities of organic liquids has increased with the trend in research toward small scale experiments. Modern autoclaves (*3, 4*) and new separation techniques give samples of 15 to 50 ml. that need further separation for analysis. Microanalytical methods such as spectroscopy can analyze minute quantities. Thus, miniature distillation is an essential step between preparation and final analysis in small scale research.

Effective miniature distillation requires more than just scaling down large columns. Although efficient columns have been designed for distilling small samples (*1, 5, 7*), less has been done in developing techniques for scaled-down operation. On the smaller scale, control of throughput and reflux ratio must be more precise; a fluctuation as little as 0.1 volt in the power to the distilling flask heater can upset column operation. Losses of sample must be avoided; distillate volumes must be read accurately and entrainment of vapors during vacuum operation must be eliminated. Contamination of fractions must be minimized; a single drop of distillate can represent 10% contamination in a succeeding fraction if it is held on the walls of the receiver system. Improved distilling units and controls would make miniature distillation a more practical technique.

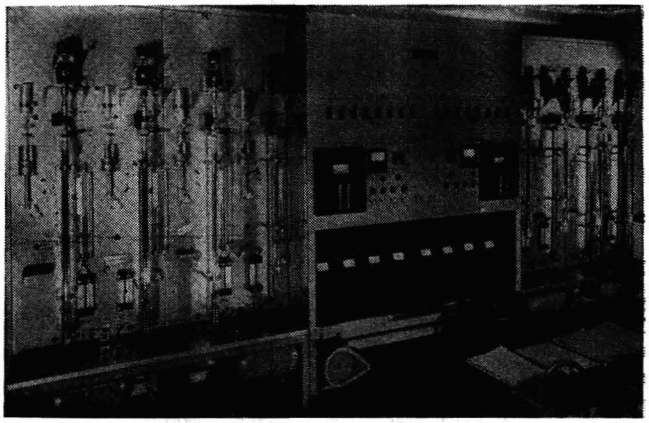

Figure 1. Installation of miniature distilling units

A fractionation installation has been designed and built that meets the needs of the small scale. The present paper describes the equipment and method of operation; a future paper will report testing and evaluation of the columns.

EQUIPMENT AND OPERATION

Figure 1 shows the installation for eight miniature distilling units, four on each side of the central instrument section. Three types of columns are used: spinning band, Hyper-Cal, and concentric tube. Each may be installed at any of the eight positions. Tanks and pumps for the vacuum systems are located below.

Distilling Units. One distilling unit, shown in Figure 2, consists of a flask, column, receiver, and accessories. Multilayer metal radiation shields in evacuated jackets surrounding the flask and column (*6*) eliminate the need for external heaters. The receiver assembly is to the left of the column. Also shown are a light-ends trap at the upper left, Dubrovin and tilting McLeod gages for measuring reduced pressures, and flowmeters at the lower right for controlling water or air to the condenser.

The jacketed 25- to 100-ml. distilling flasks (*6*) reduce thermal decomposition of samples; low heat losses permit temperatures of 350° C. with a 50-watt heater. The heater is a removable cartridge inserted from the bottom of the flask into a well. This construction gives good circulation of the sample and minimizes bumping.

The three types of columns make it possible to fractionate most organic liquids. As shown in Table I, all have heights equivalent to a theoretical plate (HETP) near 1 cm. at 20 ml. per hour throughput and total reflux. The spinning band has a low holdup and pressure drop and is especially useful at reduced pressures. The Hyper-Cal, which contains Heli-Grid packing (*7*), is used to best advantage at atmospheric pressure and where the volume of components being fractionated is sufficient so that theoretical plates, rather than holdup, is the most important factor. The all-glass concentric-tube column has an annulus of 0.7 mm. and is not attacked by corrosive liquids; it has a low HETP but requires a high reflux ratio (*8*).

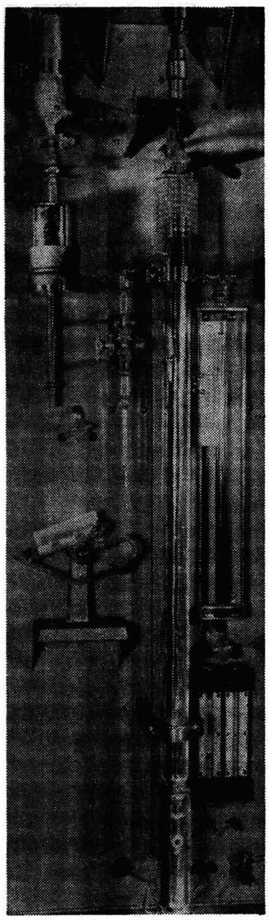

Figure 2. Distilling unit

The receiver assembly, shown in Figure 3, is designed so that distillate does not come in contact with any wall, stopcock, or grease, but falls from the drip tip through the large-bore stopcock. A male joint on the receiver allows the sample to be poured without contacting grease. The receivers, 1.5- to 2.5-ml. capacity, are calibrated in 0.02-ml. divisions.

Solid distillates are kept liquid by heating the take-off arm with resistance wire and the receiver with a small radiant heater. The heater is a semicylindrical sheet of polished metal backed with resistance wire. To permit easy changing of receivers, the heater is hinged at the top.

The head of a spinning-band column is shown in Figure 4. (Certain apparatus improvements described are the subject of pending patent applications.) The band is driven at 2000 r.p.m. through a ball-bearing–supported magnetic coupling (*2*) that eliminates the need for a stuffing box. To avoid affecting vapor temperatures, the top of the band must not be above the rectifying tube. The thermocouple is encased in hypodermic-needle tubing; to give correct readings it has to be experimentally located while refluxing a pure compound. The heads for the other columns have similar parts but differently arranged.

Table I. Typical Column Data

	Spinning Band	Hyper-Cal	Concentric Tube
Inside diameter, mm.	5	8	8
Length, cm.	90	60–90	60
Sample size, ml.	10–50	20–100	10–50
Fraction size, ml.	0.5	0.5–1.0	0.5
Throughput, ml./hour	10–30	20–30	20–30
Take-off rate, ml./hour	0.5–1.5	0.5–1.5	0.2–0.5
Reflux ratio	40:1	40:1	100:1
HETP, cm.[a]	1.1	0.7	0.9
Holdup per 90 cm., ml.[a]	1.9	4.8	3.2[b]

[a] At 20 ml. per hour throughput and total reflux.
[b] Determined on 60-cm. column.

In the column heads, condensate is prevented from leaking past the needle seat by careful alignment of the stainless steel needle (*6*) and by keeping the needle tip clean. The column head should be adjusted so that the needle is vertical and moves freely in the glass guide near the solenoid. For cleaning, the needle is removed and the tip is gently rotated on cotton impregnated with oil and polishing compound. This operation cleans rather than grinds; the original finish on the metal should not be disturbed.

In spinning-band columns, a loose-fitting or vibrating band reduces fractionation efficiency. The band should be tight enough that it does not turn freely until reflux is established; the final criterion for judging the fit of the band is plate testing. Vibration is likely to occur at speeds above 2200 r.p.m. and can occur at lower speeds if the band and column are not properly aligned.

The motors used with the spinning band are rigidly mounted and easily aligned with the magnetic drives. Direct-drive ball-bearing motors are better than those with gear or friction drives. Figure 5 shows a mounted motor. The slotted bracket permits vertical and horizontal adjustment; the rubber cushions reduce vibration. A tachometer-generator, coupled to the motor by rubber tubing, is connected electrically to a tachometer on the instrument panel.

Controls. Good separations require more precise controls than are used in large scale distillation. Especially important are controls for throughput, column pressure, and reflux ratio. The spinning-band units also require regulation of band speed.

Each half of the instrument section serves four units. Figure 6 shows the type and arrangement of the instruments. Across the top are timer clocks for controlling reflux ratio by activating the solenoid at the column head. To the left is an indicating potentiometer that measures vapor and flask temperatures up to 260° C. To the right are a pyrometer for measuring higher flask temperatures, a tachometer, and variable transformers for controlling band speed. Below these instruments on an inclined Bakelite panel are wattmeters, transformers, and switches for controlling the power to the flask heaters.

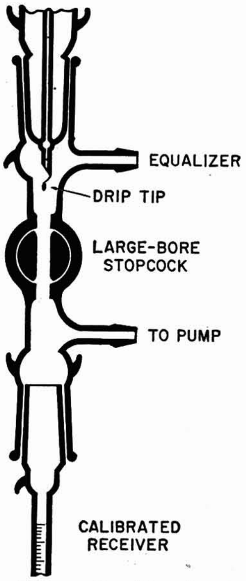

Figure 3. Receiver assembly

Column throughput is controlled by the power to the heaters. Both regulation and fine adjustment of the voltage are needed. An electronic voltage regulator, serving the eight units, controls line voltage within 0.01%. This degree of regulation is provided because neither 1.0% regulation by a constant-voltage transformer nor 0.1% by an electronic regulator was adequate. Each unit has a heater-control circuit, as shown in Figure 7. A high or low range is chosen by the three-pole double-throw switch. A base voltage is then selected, and a fine adjustment is added by the variable transformer. Because the output of the transformer is stepped down by an 18 to 1 ratio through filament transformers, the range of the fine adjustment is 6.3 volts. The smallest change that can be made is 0.015 volt. The wattmeter serves only as a rough guide for the operator.

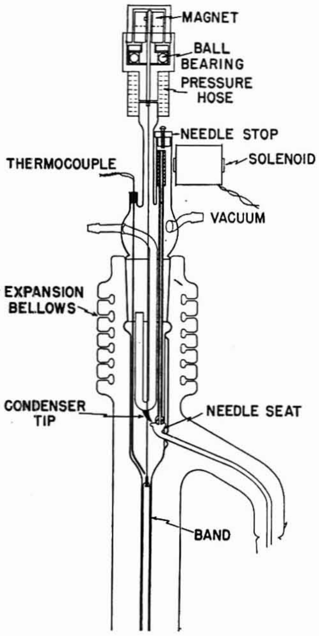

Figure 4. Head of spinning-band column

To reduce vapor losses, a closed vacuum system is used rather than continuous or intermittent pumping. Because the system is kept free from leaks, no automatic pressure regulator is needed. This design gives simpler control and better measurement and avoids oxidation of samples because no air enters the column.

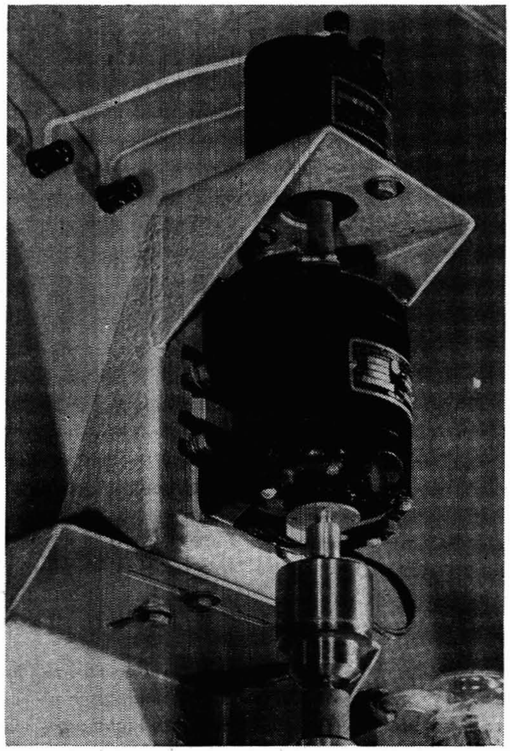

Figure 5. Motor for spinning-band column

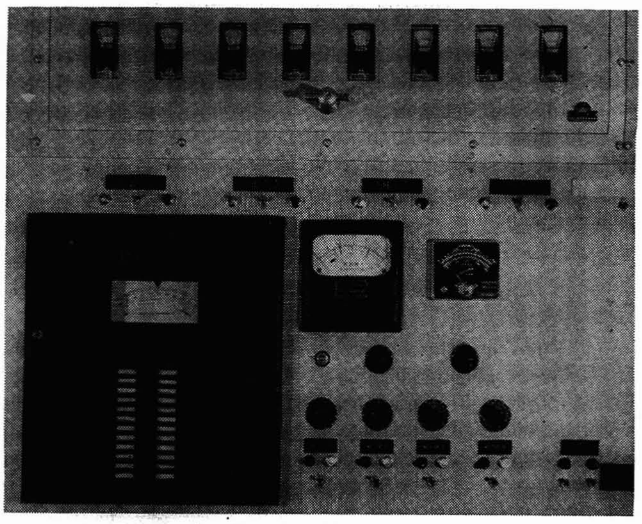

Figure 6. Detail of instrument panel

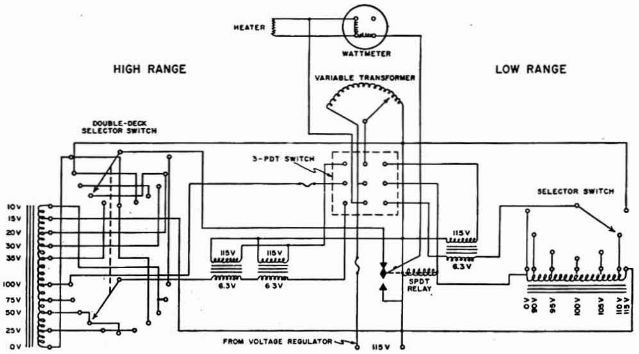

Figure 7. Circuit for a flask heater

Figure 8 shows the layout of the vacuum system. The 5-gallon surge tank is kept at the minimum pressure of the pump and serves two columns. The 2-gallon tank is set at any desired distillation pressure by the three-way stopcock below the tank. All vacuum lines are solder-sweated copper tubing; metal-to-glass connections are made by short lengths of rubber pressure tubing. Pressures from 10 to 20 mm. are measured on a Dubrovin gage and those below 10 mm. on a calibrated tilting McLeod gage.

Operation. The selection of column type and operating conditions depends on volume of the key components, degree of separation desired, distillation pressure, and corrosiveness to metals. With the sensitive controls built into the equipment, operation of the miniature units is similar to that of larger units. Columns containing fixed packing are preflooded. All are refluxed at the desired throughput for about 4 hours before beginning take-off. Throughput is measured by counting the drops from the condenser drip tips. The timer clocks, set to give the desired reflux ratio, govern take-off rate.

The attention needed by a single unit depends on the type of sample, distillation pressure, and fraction size. One operator can normally take care of four units. With take-off rates of 0.5 to 1.5 ml. per hour, a 30-ml. sample requires 20 to 60 hours of distillation time.

TYPICAL DISTILLATIONS

Miniature Hyper-Cal and spinning-band units have been applied to a wide variety of samples. Typical of the results obtained are those from the routine distillation of three different samples of hydrocarbons.

Figure 9 is a plot of the refractive-index and temperature-volume data from the distillation of 50 ml. of olefin polymer in a spinning-band column. Fractions of 0.5 ml. were taken. The sample is composed of compounds of four to more than 24 carbons. Despite the broad range of components and the use of reduced pressure, the distillation gave clearly defined data for each carbon number through 16. Larger charges of similar materials gave well-defined data above the 16-carbon range.

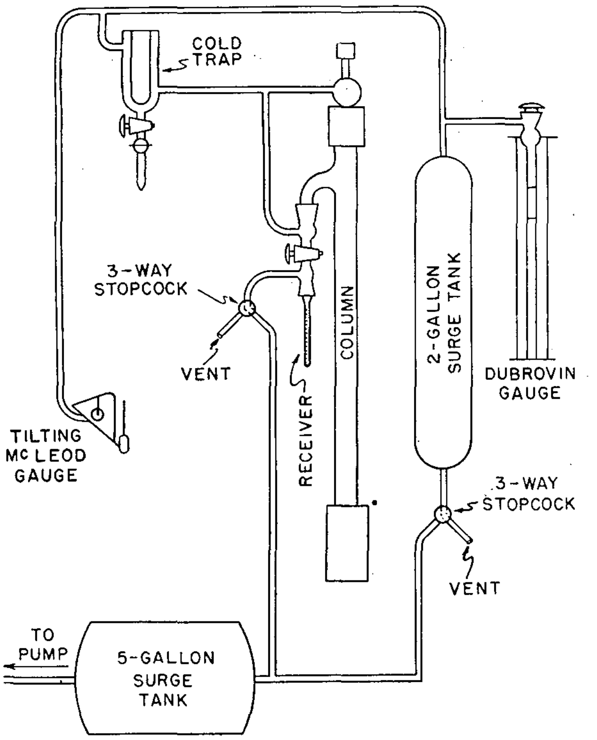

Figure 8. Vacuum system

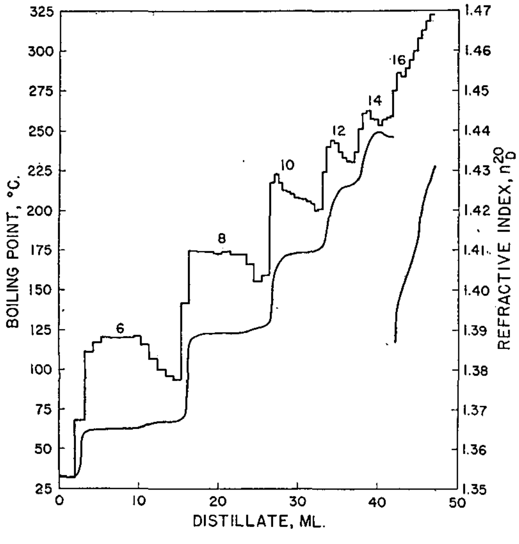

Figure 9. Distillation of olefin polymer

Numbers refer to carbon number of plateau. Throughput, 24 ml. per hour; reflux ratio, 24 to 1 to 27 ml., 48 to 1 thereafter; pressure, atmospheric to 42 ml., 20 mm. of mercury thereafter; time, 85 hours

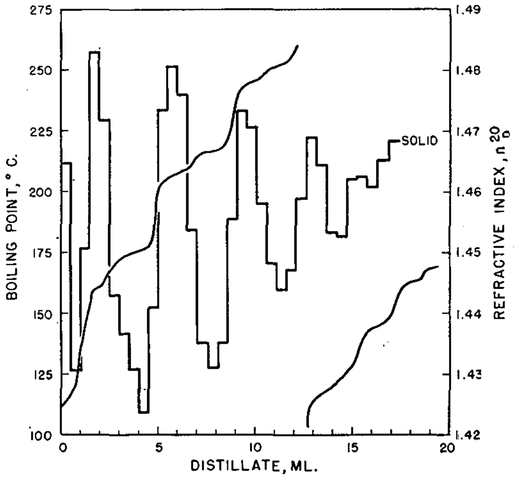

Figure 10. Distillation of aromatic concentrate

Throughput, 24 ml. per hour; reflux ratio, 48 to 1; pressure, atmospheric to 12 ml., 5 mm. of mercury thereafter; time, 52 hours

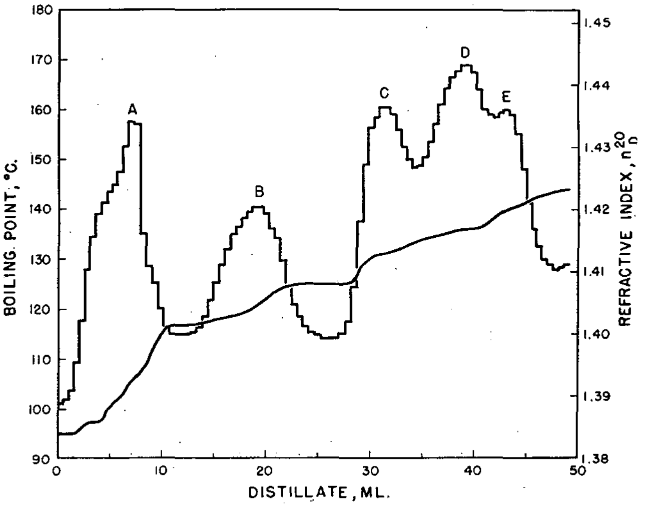

Figure 11. Distillation of 7- and 8-carbon fraction of virgin naphtha

Throughput, 28 ml. per hour; reflux ratio, 56 to 1; time, 100 hours

Figure 10 shows the results of a spinning-band distillation of only 20 ml. of an aromatic concentrate. This run demonstrates the ability of the column to concentrate components present in small quantities. The alternate refractive index peaks and valleys represent clear differentiation between aromatic and aliphatic compounds across a broad range of temperature and pressure, although some of the aromatics were present in quantities of less than 1 ml.

A Hyper-Cal distillation of a 50-ml. sample of the 7- and 8-carbon fraction of a mid-continent virgin naphtha is shown in Figure 11. This complex mixture included paraffins, naphthenes, and aromatics. Refractive-index peaks *A*, *B*, *C*, *D*, and *E* represent concentrates of toluene, 8-carbon naphthenes, ethylbenzene, *p*- and *m*-xylene, and *o*-xylene. Some estimate of the degree of separation can be realized from the fact that the toluene content of the charge was only 0.7 ml.

The average recovery in these samples was 98%, typical of routine operation.

CONCLUSION

With proper equipment, miniature distillations are as useful for analytical purposes as those done on a larger scale. Although they do not save distillation time, they increase the value of small scale experimentation. Many of the techniques and controls should be useful in other laboratories for operating a single unit. Evaluation of the usefulness of the columns will be completed by plate testing.

ACKNOWLEDGMENT

The authors thank A. G. Nerheim for the distillation data and H. M. Grubb for the design of the heater-control circuit.

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RECEIVED for review May 26, 1954. Accepted December 18, 1954. Presented before the 19th Midyear Meeting of the Division of Refining, American Petroleum Institute, Houston, Tex., May 1954.

Determination of Carboxylic Amides by Reduction to the Corresponding Amine

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Central Research Laboratory, General Aniline & Film Corp., Easton, Pa.

A method is described for determining primary, secondary, and tertiary amides based on reduction of the amides to the corresponding amine using lithium aluminum hydride. The amine formed is steam-distilled from the reaction mixture and is then titrated with standard acid. The procedure was initially developed for determining sodium *N*-lauroyl sarcosinate (*N*-lauroyl-*N*-methyl sodium glycinate). Once the procedure was established for this compound, it was tried on other samples of amides and found to work satisfactorily. The range of the method is large, extending from aliphatic amides of low molecular weight to the fatty amides. Other types of amides were also found to be determinable by the method. The accuracy and precision of the procedure are usually within ±2% and are often within ±1%, depending on the particular amide.

THE nitrogen content of a sample has been used to determine amide content (*1, 3–5, 11, 12, 14, 15*), but this approach lacks specificity for the amide grouping. Hydrolysis (*13, 14*) is mainly applicable only to some primary amides and is very seldom applicable to any secondary or tertiary amides, as these are rather difficult to hydrolyze. A colorimetric method has been devised (*2*) involving conversion of the amide to the corresponding hydroxamic acid and formation of the colored ferric chloride complex, which is measured colorimetrically. This method is excellent for determining small amounts of amides, but where the amount of amide compound in a sample is rather large (10 to 100%), a higher precision and accuracy than are obtainable by the colorimetric approach are generally needed.

Mitchell and Ashby (*8*) determined primary amides by reaction with 3,5-dinitrobenzoyl chloride. Olleman (*10*), Krynitsky (*7*), Hochstein (*6*), and Zaugg and Horrom (*16*) determined amides via active hydrogen analysis, using lithium aluminum hydride among other reagents which react with active hydrogen atoms. These methods lack in specificity, as many organic compounds contain active hydrogen atoms, and tertiary amide groups do not.

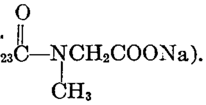
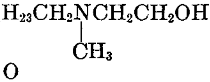
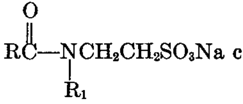
Nystrom and Brown (*9*), in an article on reactions of lithium aluminum hydride, report that the reduction of amides proceeds to the formation of the corresponding amine:

$$2RCONR_2 + LiAlH_4 \rightarrow 2RCH_2NR_2 + LiAlO_2$$

It was thought that an analytical method could be devised based on this chemical reaction, and the procedure described below was evolved.

The method is based on reduction of the amide with the hydride and steam distillation of the amine formed. It was first thought that the amine formed could be titrated in the reaction mixture. It was supposed that one break would be obtained for the strong bases (metal hydroxides) and a second break for the weaker base (amine). However, the results obtained by this approach were very high, probably because aluminum hydroxide is not so strong a base as was first supposed. In view of these results, the steam distillation step was added to the analysis.

In the determination of fatty acid amides the corresponding amines formed on reduction steam distill very slowly; so ethylene glycol was used instead of water in the distillation.

The glycol distillation is used for the determination of the sodium *N*-lauroyl sarcosinate (Medialan,  $C_{11}H_{23}C(=O)\text{—}N(CH_3)CH_2COONa$). The amine formed in the reduction of this compound is distillable from a caustic solution (the Kjeldahl distillation). The sarcosinate contains a carboxyl group that in the resultant amine should be in the form of the salt in the alkaline solution. This amino acid salt should be difficult to distill. However, it is presumed that in the reduction step, the carboxyl group is reduced to the alcohol, so that the resultant amine would be  $C_{11}H_{23}CH_2N(CH_3)CH_2CH_2OH$, which is distillable. Amides of the type  $RC(=O)\text{—}N(R_1)CH_2CH_2SO_3Na$ cannot be determined by this method. Evidently the sulfonic acid group is not satisfactorily reduced, and the salt of the amine will not distill. Very low results are obtained on these compounds.

This method is applicable to a large range of amides, as seen in Table I. Primary, secondary, and tertiary amides of low molecular weight acids and fatty acids, as well as difunctional amides, were successfully determined. A cyclic amide (methyl pyrrolidone) was also determined. Unsatisfactory reductions were noted for acrylamide, *N-tert*-butyl acrylamide, and urea. In the case of *N,N*-diphenyl acetamide, the resulting amine (diphenylethylamine) is too weakly basic to titrate, even in solvents designed for titrating weak bases.

Many functional groups react with lithium aluminum hydride, but very few result in the formation of a volatile base. Nitriles, imides, and aliphatic nitro compounds are the only others which, to date, are known to form an amine on reduction with the hydride The aliphatic nitro compounds are rarely found in amide samples; nitriles are sometimes found in samples of primary amides.

Lithium aluminum hydride will reduce nitriles to the corresponding amine, and this will result in high values for the amide. The reduction of nitrile does not proceed to completion in all cases of nitriles; therefore, this would not be a good general method for nitriles. Benzonitrile, butyronitrile, capronitrile, and chlorobenzonitrile were determined satisfactorily by this approach (see Table II). Nitriles which were tried and could not be reduced completely to amine in a convenient length of time were acetonitrile, acrylonitrile, succinonitrile, adiponitrile, phenylacetonitrile, 3-butenenitrile, γ-phenoxybutyronitrile, lactonitrile, *m*-nitrobenzonitrile, and 1-naphthonitrile.

Table I. Results

Compound	%	% by N	Procedure
Acetyl diethylamine (*N*,*N*-diethylacetamide)	99.2 98.1 98.2 98.4 98.4	97.7	A
N-butyramide	95.4 97.2 96.2	97.1	A
N-methyl acetamide	93.5 92.8 93.8 94.2	95.2	A
N-methyl pyrrolidone	98.2 98.6 98.1	97.8	A
Acetamide	100.6 100.8 100.3	99.8	A
Propionamide	101.1 100.8 100.3 100.0 100.0	100.4	A
γ-Hydroxybutramide	96.3 96.0 95.8	96.1	A
Methyl glycolamide	95.6 95.2 95.4	96.3	A
N,*N'*-dimethyl oxamide	99.9 99.8 99.5 99.8	100.8	A
Benzamide	98.5 100.0 99.7	100.8	A
Formamide	99.0 98.9 98.6	100.6	A
Dimethyl formamide	90.7 89.5 92.2	93.4	A
Octanamide	96.4 98.1 97.5	96.6	A
Hexadecanamide	95.2 94.6 94.6 94.9	95.1	B
Methyl stearamide	97.4 98.6 100.0 98.0	98.3	B
Stearamide	92.4 92.7 93.7	94.5	B
Dodecanamide	96.0 95.8 94.2	96.6	B
Methyl dodecanamide	94.4 92.3 93.1	97.6	B
Succinimide	99.3 99.9	98.8	A
N-lauroyl sarcosine	100.5 100.2 100.3 99.8	99.1	B[a]
Sodium *N*-lauroyl sarcosinate	30.27 30.11 30.04	. . .	B[a]
Sodium *N*-lauroyl sarcosinate[c]	94.7 93.7 93.7 94.6 94.4	95.8	B[a]

[a] Reflux time for reduction step was increased to 1.5 hours for these compounds.

[b] A 30% aqueous solution of sodium *N*-lauroyl sarcosinate. Each sample was dried at 100° C. before reduction

[c] Sample contained 5.7% water as determined by Karl Fischer titration.

It is advisable, therefore, if nitriles are suspected in a sample of a primary amide, to use the procedure of Mitchell and Ashby (*8*) for determining the primary amide. Nitriles seldom occur in secondary and tertiary amides, so that possible interference is minimized.

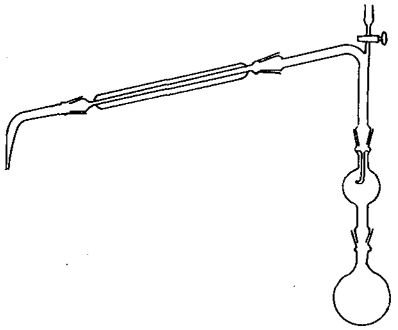


Figure 1. Distillation apparatus

Compounds that contain active hydrogen atoms as well as alkyl halides, esters, epoxides, and azoxy compounds will consume hydride. If such compounds are present in quantity, enough hydride must be present in the reaction mixture to convert all the amide to amine. It is impossible to run dilute aqueous solutions of amides by this method, as too large an excess of reagent would be needed. However, samples containing 10% water were successfully run by this method. Samples containing 50% water showed results which were 10% low in amide, but using more hydride reagent may make it possible to run 50% aqueous solutions. It may be possible to run samples containing 25% water, but this was not tried.

The precision of the analysis described below is usually within ±2% and very often within ±1% for the various amides tried. The accuracy is about the same as the precision; however, in many of the amides tried the precision of the nitrogen analysis (Dumas) used to assay the amides is poorer than the precision of the reduction method being tested. For the amides of nitrogen content below 10%, the accuracy values are controlled not by the reduction method but by the nitrogen method (the Dumas nitrogen analyses are reproducible to ±0.2% of nitrogen).

The analysis is very simple to carry out and the apparatus is, for the most part, standard laboratory equipment. Precautions are required in handling lithium aluminum hydride; these are described on every package of the material. It is well not to use a hydride sample that is too old or that has been stored in a loosely stoppered container; it may be too hydrolyzed to be effective.

REAGENTS

Lithium aluminum hydride. Ten grams of lithium aluminum hydride are refluxed with 500 ml. of anhydrous diethyl ether for several hours. If the hydride is finely divided, it will dissolve in a relatively short time. Insoluble products, formed by the reaction of impurities in the ether with the lithium aluminum hydride, settle on cooling, and the clear solution can be pipetted off as needed. The solution should be protected from atmospheric moisture. The usable life of the solution is about one month.

Standard 0.02*N* sulfuric acid.
Standard 0.02*N* sodium hydroxide.
6*N* sodium hydroxide.
Methyl purple indicator (Fleisher methyl purple, Burrell Corp., Pittsburgh, Pa.).
Ethylene glycol.
Isopropyl alcohol.

DISTILLATION APPARATUS

The distilling apparatus used in Procedure A is the standard Kjeldahl steam distillation equipment.

The distilling apparatus in Procedure B consists of a 200-ml.

Table II. Nitrile Results

Compound	%	% by N	Procedure
Benzonitrile	98.90 98.69 98.81	98.67	A
Butyronitrile	96.15 96.40 96.72	98.86	A
N-capronitrile	98.45 99.07 97.76	99.65	A
p-Chlorobenzonitrile	99.95 98.93 99.76	102.06	A

round-bottomed flask connected to a Kjeldahl bulb which is attached to a water condenser by a 75° connector. A stopcock and funnel are sealed on the connector at the bend so that ethylene glycol can be dropped into the flask (see Figure 1).

PROCEDURE A

An exactly weighed sample containing approximately 0.0006 mole of amide is placed in a 100-ml. Kjeldahl flask, and 5 ml. of lithium aluminum hydride reagent are added. The solution is allowed to stand for 15 minutes at room temperature to ensure complete reduction of the amide, and the flask is then attached to the Kjeldahl distillation apparatus. A 200-ml. Erlenmeyer flask containing exactly 50 ml. of 0.02*N* sulfuric acid is placed on the apparatus, so that the end of the condenser is below the surface of the acid. Water is added dropwise to the reaction flask until the excess lithium aluminum hydride is decomposed. Ten milliliters of 6*N* sodium hydroxide are added and steam distillation is carried out as in a Kjeldahl determination. About 50 ml. of distillate are collected in the 0.02*N* sulfuric acid and the excess acid is titrated with standard 0.02*N* sodium hydroxide to the green end point of methyl purple indicator. Per cent amide is calculated as follows:

$$\frac{(\text{Titration for 50 ml. of acid} - \text{titration for sample}) \times N \text{ of NaOH} \times \text{M.W. amide} \times 100}{\text{Weight of sample} \times 1000} = \% \text{ amide}$$

PROCEDURE B

A weighed sample containing approximately 0.0006 mole of amide is placed in a 200-ml. round-bottomed flask, and 10 ml. of lithium aluminum hydride reagent are added. The mixture is refluxed on a steam bath for 0.5 hour. The flask is cooled to room temperature and the excess reagent is decomposed by dropwise addition of water. After the reagent is completely decomposed, the sides of the flask are washed with about 10 ml. of water, and 5 ml. of 6*N* sodium hydroxide are added. A few boiling chips and 25 ml. of ethylene glycol are added before the flask is attached to the distilling apparatus. The solution is distilled at a rapid rate nearly to dryness and 25 ml. of ethylene glycol are added through the stopcock on the connector at such a rate that boiling does not stop. The addition and distillation of 25-ml. portions of ethylene glycol are continued until 100 ml. have been distilled. The condenser is washed with approximately 50 ml. of hot isopropyl alcohol, and the amine contained in the distillate and washings is titrated potentiometrically with 0.02*N* sulfuric acid. Per cent amide is calculated in the following manner:

$$\% \text{ amide} = \frac{\text{ml. of } H_2SO_4 \times N \text{ of } H_2SO_4 \times \text{M.W.} \times 100}{\text{weight of sample} \times 1000}$$

ACKNOWLEDGMENT

The authors would like to acknowledge the contribution of Thomas Bonstein, who ran the nitrogen analyses on the samples used in this study.

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Received for review October 22, 1954. Accepted December 18, 1954.

Polarographic Determination of Traces of Fluoride and Iron

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A method of analysis was required for microgram quantities of fluoride and iron in the presence of radioactive substances. Active oxygen and peroxides are formed in aqueous solutions as a result of intense radioactivity. These active substances attack dye and fluorescent salts used in many analytical methods for fluoride. A polarographic method, using rotating platinum microelectrodes, was developed in which the fluoride ion forms a complex with ferric iron and the iron-fluoride complex is reduced irreversibly at potentials more negative than the reduction of ferric iron. The dissociation constant of FeF^{++} was determined and found to be 6.9 × 10^{-6}. The method is suitable for determination of microgram quantities of fluoride and iron in solutions containing radioactive substances. One microgram of fluoride and 0.33 microgram of iron may be determined with an accuracy of about 15%.

BECAUSE there were few satisfactory methods available, an analytical procedure for the determination of microgram amounts of fluoride and iron in the presence of high-level radioactivity has been developed. Many of the microgram analytical methods reported for determination of fluoride depend on a bleaching action of fluoride on a colored or fluorescent metal-organic salt (*15, 22*). These methods are not accurate because of insensitivity of the end point, but some improvement in them has been made by using a spectrophotometer (*6*) or fluorometer (*17, 23*) to determine the end point. Other amperometric titrations for fluoride could not be used on a microgram scale because of limitations of solubility or interferences (*3, 4, 16, 20*).

Active oxygen and peroxides are formed in aqueous solutions as a result of intense radioactivity (*8*). This active oxygen attacks the dye in colorimetric methods for fluoride analysis, caus-

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ing an error in results. The polarographic methods for fluoride and iron described here are not affected by oxygen or peroxides because ferric iron is more easily reduced than is oxygen.

The concentration of ferric iron in a solution is measured polarographically by using a rotating platinum wire microelectrode to reduce ferric iron to ferrous iron. As fluoride ion is added to the iron solution, a complex is formed which is reduced irreversibly at potentials more negative than ferric iron. The decrease in the concentration of ferric iron is related to the amount of fluoride ion added for analytical purposes. Substances which form equally stable complexes with iron must be eliminated. The fluoride frequently must be distilled from insoluble compounds. It was found that a borosilicate glass microstill was satisfactory, indicating that silica did not interfere directly in the analysis.

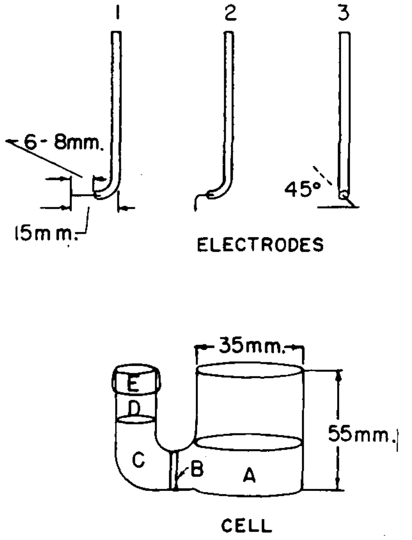

Figure 1. Electrodes and polarographic cell

A. Sample, 10 ml.
B. Coarse glass filter plate
C. Agar salt bridge of saturated KNO_3
D. Saturated KNO_3 solution
E. Paraffin coating

The rotating platinum wire microelectrode has been used to indicate the end point of a titration with silver nitrate or other reducible substance (*9, 10, 13*). One advantage of the platinum electrode is that it is useful in studying redox potentials in the range where mercury would be oxidized. The diffusion current, which is proportional to the concentration of the measured ion, is a function of the speed of rotation of the platinum electrode. In the method described the rotating speed of the electrode is adjusted to expand the polarographic wave to the full scale of the recorder of the polarograph, thus increasing the sensitivity of the determination. A constant speed of rotation of the electrode is difficult to maintain; generally, synchronous motors with a gear reduction drive are used. In the present application a Sargent cone-drive induction motor with a constant voltage source functions satisfactorily. The adjustable speed knob is clamped securely after the desired speed is obtained. Daily calibration of the electrode is required because of slight wear of the rubber O-ring on the cone reduction-drive.

REAGENTS AND APPARATUS

Reagents. Calcium fluoride was prepared by reaction of calcium nitrate and sodium fluoride. The precipitate was centrifuged and washed several times by decantation and centrifugation with 1% ammonium nitrate solution. It was then dried at 120° C. and ignited at 500° C. for 3 hours.

Standard iron solutions were prepared from electrolytic iron rods obtained from Johnson, Matthey and Co., Ltd., London, through Jarrell-Ash Co., Boston, Mass. A weighed sample of iron was dissolved in concentrated hydrochloric acid, and diluted to 100 ml. A 10-ml. aliquot was evaporated to near dryness several times with concentrated nitric acid, and diluted to 100 ml. Tests of the diluted sample for chloride ion were negative.

Lanthanum fluoride was prepared according to the method of Hirsch (*5*). Analysis gave the following results: lanthanum found, 70.12%; calculated, 70.91%; fluoride found, 28.96%; calculated, 29.09%.

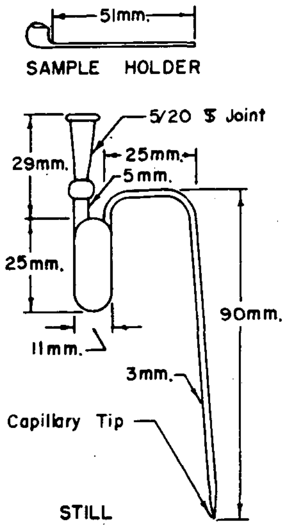

Figure 2. Distillation apparatus

Lead chlorofluoride was prepared according to a modified method of Starck (*21*). An equivalent amount of sodium fluoride solution was added to a saturated lead chloride solution and the precipitate was filtered, washed, and dried at 120° C. for 20 hours.

Analytical reagent grade sodium fluoride, dried at 110° C. for 12 hours, was used.

Apparatus. An Electrochemograph Type E polarograph was used to record the polarograms. The platinum wire microelectrodes are illustrated in Figure 1. The three electrodes differed from those previously described (*10, 14, 19*) in that they moved through the solution in a different manner. These electrodes consist of a piece of smooth and new 20-mil platinum wire sealed into 6-mm. soda-lime glass tubing. It is desirable to round off the exposed end of the wire with fine emery cloth; sharp edges and points must be removed because oxidation occurs more easily from these portions than from a smooth surface. The glass-platinum seal is made in an oxidizing flame. Any cracks or imperfect seals at the tip of the glass-platinum seal cause noticeable effects on the polarogram. The platinum wire must be bent with care near the seal of electrodes of Types 2 or 3 of Figure 1. A recalibration is necessary if the shape of the electrode is changed in any way. The electrodes are designed so that the platinum wire moves through the solution perpendicular to its axis. By moving in this manner the wire continually comes in contact with fresh portions of solution, resulting in constant diffusion conditions. The electrodes are designed so that there is no exposed wire near the center of rotation of the electrode where unsteady diffusion conditions exist.

The platinum microelectrode is washed with concentrated hydrochloric acid and placed in 10% sodium cyanide solution for such a time as is necessary for the individual electrode to give a background current of the order of 0.1 μa. at 0.2 volt measured against a saturated calomel electrode. If the electrode is smooth and bright, only a few hours are required to condition it in the sodium cyanide solution. If the electrode is not properly prepared, the reduction polarogram of ferric iron deviates considerably from reversible behavior, an oxygen reduction wave appears, and erratic behavior is observed. The electrodes are cleaned frequently in 10% sodium cyanide solution, especially if potentials more negative than 0.2 volt are to be used. The surface of the platinum appears to change owing to discharge of hydrogen, and sodium cyanide solution dissolves enough of the platinum to present a new inert surface to the solution.

The polarographic cell is shown in Figure 1. A saturated calomel electrode is used as a reference electrode (*7*). The arm of the reference electrode is placed in the side arm of the cell. The top of the side arm, *D*, was coated with paraffin to prevent the potassium nitrate solution of the salt bridge from establishing electrical contact with the constant temperature bath. When this happened, stray currents were introduced into the polarographic circuit.

The glass distillation apparatus shown in Figure 2 was suggested by a platinum still described by Watters and coworkers (*18*). An aluminum heating block 1.5 inches in diameter is provided to hold the still, and is electrically heated by resistance wire wrapped around the block on asbestos insulation. The wire is covered by asbestos and Sauereisen cement. Power required to heat the aluminum block is about 200 watts. Temperature is measured by a thermometer in a hole of the block 0.5 inch from the sample.

Table I. Effect of pH on Half-Wave Reduction Potential and Diffusion Current[a]

pH	Half-Wave Potential, Volt *vs.* S.C.E.[b]	Diffusion Current, μa.
0.301[c] (0.5N HNO_3)	0.473	13.6
0.699[c] (0.3N HNO_3)	0.486	13.6
1.0[c] (0.1N HNO_3)	0.495	14.0
1.15	0.489	13.5
1.75	0.487	13.7
2.0	0.486	13.6
2.3	0.487	14.0
3.1	0.435	13.5
3.7	0.426	3.3

[a] Solution $1.063 \times 10^{-4}M$ Fe(III) in 0.1N KNO_3.
[b] Resistance of cell 600 ohms.
[c] No potassium nitrate present in these experiments.

Table II. Ratio of Diffusion Current to Concentration of Iron(III)[a]

Concentration Iron(III), Mole/Liter	Diffusion Current, μa.	Ratio, $\frac{\text{Diffusion Current}}{\text{Concentration}}$	Half-Wave Potential[b], Volt *vs.* S.C.E.	Reversibility Test Slope of Curve, E *vs.* log $\frac{I}{I_D - I}$
1.063×10^{-5}	1.54	1.453×10^{5}	0.503	−0.057
5.31×10^{-5}	7.75	1.46×10^{5}	0.494	−0.065
1.063×10^{-4}	15.4	1.45×10^{5}	0.496	−0.063
5.31×10^{-4}	76.5	1.44×10^{5}	0.490	−0.0695

[a] In 0.1N potassium nitrate, 0.01N nitric acid.
[b] Corrected for IR drop (resistance of cell 600 ohms).

EXPERIMENTAL

Electrodes. Some characteristics of the oxygen reduction wave in 0.1N potassium nitrate and 0.01N nitric acid were studied. It was found that the wave was reproducible with a half-wave potential of 0.078 volt (versus the saturated calomel electrode) when the electrode was cleaned after each polarogram. If hydrogen was discharged on the platinum electrode during the preparation of a polarogram and the electrode was not cleaned before the next polarogram, the oxygen wave was larger and displaced to a more positive potential. The height of the displaced oxygen wave was not reproducible and appeared to vary with the amount of current flowing through the electrode.

Distillation of Fluorides. The borosilicate glass still shown in Figure 2 was satisfactory and no indication of silica interference was noted. The finely divided bubbles produced at the capillary tip of the still delivery tube have a high surface to volume ratio and all of the fluoride is dissolved in the receiving solution.

Various times and temperatures of distillation were tried, using both perchloric and sulfuric acids in the still to decompose the insoluble fluorides. Concentrated perchloric acid starts to distill at about 140° C., while 70% sulfuric acid does not distill until about 180° C. Satisfactory and consistent distillations were made at a temperature of 150° C. for 2 hours. The time necessary to distill the fluoride was prolonged many hours at a temperature of distillation below 150° C. To avoid any distillation of acid, 70% sulfuric acid was used.

Polarographic Study of Iron. The polarographic reduction of ferric iron was studied, and a reversible reduction was observed. Table I shows the effect of different acidities on the half-wave potential and diffusion current. As the pH increases above 2.3 the half-wave potential becomes more negative, thereby indicating hydrolysis. A pH of 3 is near the upper limit for a satisfactory polarogram of ferric iron at $1 \times 10^{-4}M$ and the diffusion current decreases rapidly above a pH of 3.1. At high nitric acid concentrations the half-wave potential of the iron is shifted to a slightly more negative value, indicating weak complexing.

Experimental results tabulated in Table II indicate that the ratio of diffusion current, I_D, to concentration of iron, C, was found to be constant within experimental error. The resistance, R, of the cell was 550 to 600 ohms and all potential measurements tabulated in Table II were corrected for IR drop. The polarograms were tested for reversible behavior according to the usual procedure (*11*). Values of the slope of the curve of log $\frac{I}{I_D - I}$ versus potential E deviated only slightly from the theoretical value of 0.059 which corresponds to a 1-electron reduction.

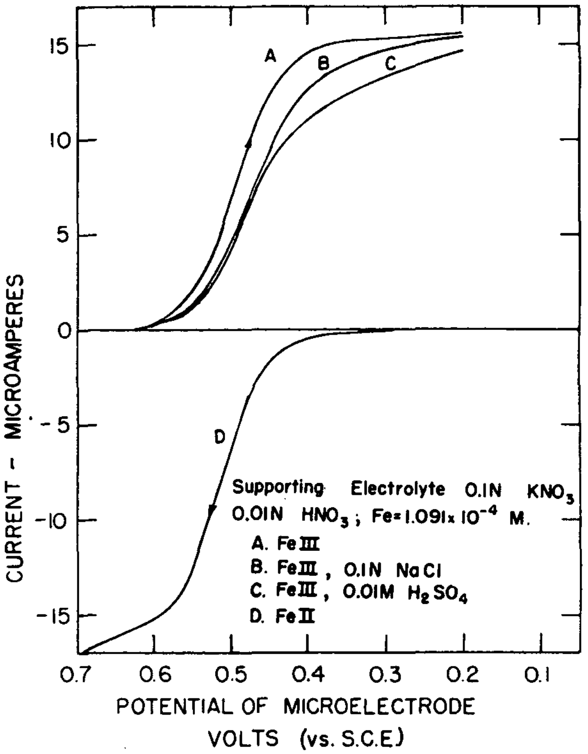

Figure 3. Polarograms of iron

The effect of sulfate and chloride on the reduction polarogram of ferric iron was studied (Figure 3). The half-wave potential of ferric ion reduction was shifted to a more negative value but the reduction was not reversible. Sulfate has more of an effect than chloride and as the sulfate and chloride concentrations increase it becomes increasingly difficult to estimate the correct diffusion current plateau. Chlorides at a concentration greater than $5 \times 10^{-2}M$ (170 mg. of chloride per 100 ml.) and sulfates at a concentration greater than $5 \times 10^{-4}M$ (5 mg. of sulfate per 100 ml.) show definite interference with the polarogram of $1 \times 10^{-4}M$ iron (0.56 mg. of iron per 100 ml.).

A significant amount of iron (about 1 to 2%) is reduced during a polarogram owing to the large reduction currents. A comparison of the diffusion coefficient of ferrous iron to ferric iron was made. A ferrous sulfate solution approximately 0.001M was prepared and a 10-ml. aliquot of the ferrous solution was oxidized to ferric iron by evaporating to near dryness with nitric acid. The residue was diluted to 100 ml. in the usual manner with inert electrolyte and nitric acid. A similar aliquot was not oxidized before dilution. The 100 γ of sulfate were not sufficient to cause interference with the iron polarogram. Polarograms of the two solutions indicated that the diffusion coefficients of ferric and ferrous ions were the same in the inert electrolytes used (Figure 3, curves A and D). The half-wave potentials of the oxidation and the reduction reactions agree, showing a reversible reaction. The arrows on the curves indicate how polarograms were made.

Variation of diffusion current as a function of temperature was studied. The data show a 2.5% change in the diffusion current for each degree change in temperature at 25° C.

In Figure 4 the diffusion current is shown as a function of the speed of the electrode. The variable speed drive was adjusted so that the polarographic wave was expanded to the full scale of the recorder, thereby increasing the accuracy and sensitivity of the estimation of the wave height. In general, speeds above 600 revolutions per minute are to be preferred to slower speeds because the variation in diffusion current is smaller with a change in speed. The full scale of the recorder was used for a concentration of 1 × $10^{-5}M$ iron, which corresponds to approximately 5.6 γ of iron in 10 ml. of solution, so that a polarographic error of one half of a division at 80 divisions or 0.62% is equivalent to 0.035 γ of iron.

Polarographic Study of Iron-Fluoride Complexes. When fluoride ion is added to a ferric iron solution part of the iron is complexed, so that the height of the iron wave is lowered (Figure 5). The complex is reduced at more negative potentials, but not reversibly. The FeF^{++} complex is formed first, followed by some FeF_2^+ at higher concentrations of fluoride.

Since hydrofluoric acid is a weak acid, a pH as high as possible is desirable to increase the fluoride concentration. If the pH is too high, however, ferric iron does not reduce properly at the electrode. For the present work a pH of 2 was used. It is not desirable to use buffers because competitive complexing of fluoride or iron would occur, decreasing sensitivity of the method.

Data from experiments in which a known amount of fluoride was added to a known amount of iron were plotted as calibration curves such as are shown in Figure 5. These calibration curves were found to be independent of the shape and speed of the electrode within tested limits.

The optimum range of fluoride concentration for each iron concentration was estimated and is tabulated in Table III. It is not necessary to prepare exact iron standards for the determination of fluoride if standard fluoride samples are properly calibrated against the iron solutions used.

The dissociation constant of FeF^{++} was calculated by a method similar to that of Babko and Kleiner (*1*). Results of such calculations, using data from the calibration experiments, give a value of $K = 6.9 \times 10^{-6}$. The value agrees with 5×10^{-6} reported by Babko and Kleiner (*1*) and 7.0 reported by Brosset and Gustaver (*2*). Kury (*12*) neglected the contribution of HF_2^- and found $K = 3.7 \times 10^{-6}$, which compares to 4.0×10^{-6} calculated similarly. The assumptions made in the present work are:

1. FeF^{++} is the first complex formed when there is an excess of ferric iron present.
2. There is no complexing of iron by nitrate media. (Changes were made in the nitrate concentration, but the half-wave potential changed only slightly, Table II.)
3. Ferrous ion is not affected by fluoride ion.
4. The dissociation rate of FeF^{++} is negligible compared with its diffusion rate. This assumption is supported by the invariance of the ratio of wave heights with rotation rate.

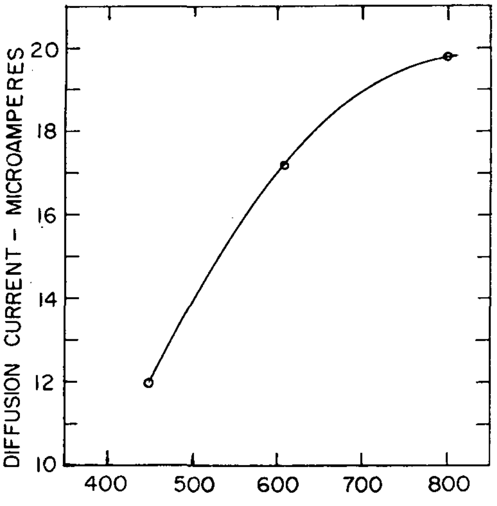

Figure 4. Effect of speed of electrode on diffusion current

Table III. Optimum Range for the Determination of Fluoride

Range Fluoride Analysis, γ	Optimum Range Fluoride Analysis, γ	Iron(III) Concentration, Mole/Liter	Estimated Error, %
Below 1	...	1×10^{-5}	12
1–2	...	1×10^{-5}	7–12
2–8	...	1×10^{-5}	3–7
8–20	10	1×10^{-5}	<3
7–47	20	5×10^{-5}	<3
11–64	28	1×10^{-4}	<3
21–130	57	2.5×10^{-4}	<3
33–200	100	5×10^{-4}	<3

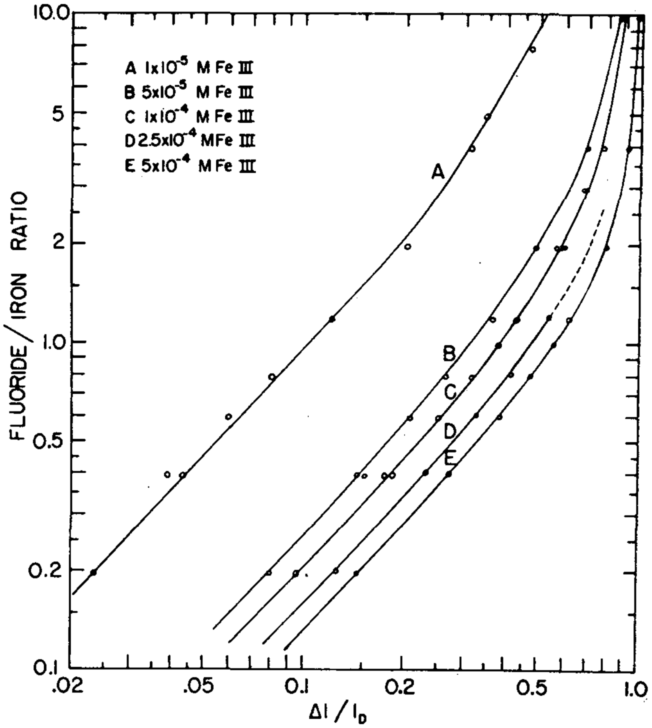

Figure 5. Calibration curves

Calculation of the free energy of formation ΔF of FeF^{++} was made by the equation

$$\Delta F = -RT \ln K = 56.6 \text{ kcal./mole} \quad (1)$$

An attempt was made to calculate the dissociation constant of FeF_2^+, but it was unsuccessful.

METHODS

Procedure for Iron. Iron can be determined in the presence of many other cations, since only a few of these can be reduced at a potential as positive as ferric iron. Ferrous iron should be oxidized to ferric iron before determination. If complexing agents are present in relatively large amounts, iron should be precipitated as iron hydroxide, collected on a microfilter, and redissolved directly in 10 ml. of 0.1*N* potassium nitrate, 0.01*N* in nitric acid. The solution need not be transferred quantitatively to the cell unless an internal standard is used. As the current is proportional to the concentration of iron, a comparison of the current of the unknown sample with a standard solution of iron can be converted to concentration of iron in the unknown.

Procedure for Fluoride. The still and sample holder are shown in Figure 2. The sample holder is used as a weighing bottle and assures the placing of the sample in the bottom of the still. The still is placed in the aluminum heating block and 0.5 ml. of 70% sulfuric acid is pipetted into the still. The standard-taper glass joint is lubricated with sulfuric acid.

An absorbing solution consisting of 10 ml. of a standard iron solution containing 0.1*N* potassium nitrate and 0.01*N* nitric

Table IV. Results of Analyses for Fluoride

Compound	Sample Weight, γ	Fluoride, γ	Fluoride Recovered, γ	Error, %
NaF		38	37	− 2.5
		19	19	0
		19	19	0
		19	19.3	2
		19	18.9	− 0.6
		19	19.1	0.5
		19	19.1	0.5
		3.8	3.95	4.0
		1.9	1.75	− 8
		1.9	1.9	0
		0.95	0.826	−13
		0.95	0.721	−24
PbClF	442	32.1	30.9	− 3.5
	420	30.5	29	− 4.8
	520	37.8	37.9	0.3
	515	37.4	34.1	− 9.0
CaF_2	180	87.5	78	−11
	117	57	57	0
	173	84.1	84.9	1.0
	228	111	110	− 1.0
LaF_3	213	62	62.7	1.0
	154	44.8	45.8	2.0

Table V. Interferences in Fluoride Analysis

Compound	Fluoride, γ	Interference in Still	Fluoride Recovered, γ	Error, %
NaF	18.5	0.4 mg. boric acid	11.6	37.3
	18.5	0.4 mg. boric acid	11.6	37.3
	18.5	0.4 mg. $NaH_2PO_4H_2O$	18.5	0
	18.5	0.4 mg. $NaH_2PO_4H_2O$	18.5	0

acid is pipetted into a test tube (13 mm. internal diameter × 85 mm.). The test tube is placed in a beaker of cracked ice, and the capillary tip of the still is inserted into the absorbing solution to the maximum depth. A slow continuous stream of air is passed through the still over the acid to sweep the volatilized fluoride into the absorbing solution. Carbon dioxide is removed from the air by an Ascarite tube. A plastic test tube is recommended for the absorbing solution.

The aluminum block is brought to a temperature of 150° C. and the distillation is continued for 2 hours. After completion of the distillation the exit tube of the still is washed free of any traces of fluoride. A rubber bulb at the standard taper joint is used to draw the absorbing solution as far as possible into the exit tube of the still several times. The absorbing solution is transferred into a clean, dry polarographic cell (Figure 1). A quantitative transfer is not necessary, since concentration only is measured. The cell is immersed in a constant temperature bath maintained at 25.1° C. Polarograms are made in the potential range 0.7 to 0.2 volt versus the saturated calomel electrode as soon as possible after distillation if glass containers are used.

To determine the amount of fluoride in a sample from the polarogram, the ratio $\Delta I/I_D$ is calculated, where I_D is the diffusion current of the standard iron solution and ΔI is the decrease in diffusion current caused by the complexing of iron by fluoride. From a set of calibration curves (Figure 5), the ratio of $\Delta I/I_D$ is converted to a ratio of fluoride to iron present, then micrograms

$$F^- = \text{normality Fe}^{+++} \times \frac{[F^-]}{[Fe^{+++}]} \times \frac{10}{1000} \times 19 \times 10^6$$

Analyses of both soluble and insoluble fluorides are summarized in Table IV. The errors are in the range predicted in Table III.

A few interferences in the fluoride determination were tested with the results tabulated in Table V. Large amounts of borates show some interference. Only relatively large amounts of chlorides (over $0.05N$ or 17 mg. per 10 ml. of absorbing solution) interfere in the determination unless removed by precipitation. There is little chance of sulfuric acid distilling if the temperature is properly controlled. The absorbing solutions containing fluoride cannot be allowed to stand in glassware for several hours.

DISCUSSION OF RESULTS

The method for iron analysis is more accurate on a molar concentration basis than the method for fluoride because of the indirectness of the fluoride determination. Larger platinum electrodes and smaller volumes of solution should allow the iron and the fluoride determination to be scaled down further. There appears to be no reason why iron cannot be determined in a large variety of inert electrolytes. An internal standard which involves the addition of a known amount of iron to an unknown allows the direct calibration of the electrode.

If polarograms are made in the potential range more negative than 0.2 volt (S.C.E.) the cell should be provided with an inert atmosphere. A cap with a hole of sufficient size so that the rotating electrode will not rub may be used. A tube providing for the introduction of nitrogen or other inert gas may be sealed onto the cell or the cap. Stirring action of the electrode is sufficient to remove oxygen from the solution.

The polarographic analysis has several advantages over some of the other methods of analysis. Rapid, relatively accurate analyses for traces of fluoride and iron ion have been developed. The method has relatively few interferences. A direct formation of iron fluoride complex is measured in the fluoride analysis, whereas in the colorimetric and some of the other microgram methods, a competition between dye and fluoride ions exists. The polarographic method is not affected by active oxygen or peroxides which may be formed as a result of radioactivity.

ACKNOWLEDGMENT

The author wishes to acknowledge the helpful suggestions and criticisms of E. F. Joy.

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Received for review February 17, 1954. Accepted December 9, 1954. Presented before the Division of Physical and Inorganic Chemistry at the 125th meeting of the American Chemical Society, Kansas City, Mo. Mound Laboratory is operated by Monsanto Chemical Co. for the U. S. Atomic Energy Commission under Contract AT-33-1-GEN-53.

Chromatographic Determination of Carboxyl Groups in Filter Paper

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A chromatographic method for determining the carboxyl groups in filter paper was developed. Experiments were carried out with several kinds of metal ions, making use of the strong bond of different metal ions to the carboxyl groups of the paper. Lead(II) ions gave the most satisfactory results. The influence of ester and lactone groups on the carboxyl group determinations was studied. The method is rapid and reliable.

THE ion exchange properties of filter paper may invalidate the results of analytical procedures involving filtration through filter paper (*12, 23*)—for example, in the determination of the solubility of radium sulfate. On the other hand, Schute (*24*) has taken advantage of the ion exchange property of filter paper in the chromatography of alkaloids in aqueous media. While Schute used papers of ordinary varieties, others (*14, 31*) have used paper especially enriched in carboxyl groups for chromatographic separations. Because of the wide use of such papers, a rapid method for the determination of carboxyl groups in filter paper is of considerable practical interest.

Except for the determinations involving infrared spectroscopy (*5*) and decarboxylation (*29*), all previous carboxyl group determinations are based on ion exchange. The ions present are usually exchanged for hydrogen ions, which are determined by titration, preferably in the presence of electrolyte, in order to avoid difficulties arising from Donnan equilibria (*18*). Neutral alkali salts are suitable for this purpose (*9*), although the end point shows considerable drift, owing to the presence of the cellulose. This can be avoided by the use of an excess of alkali (*18*). It is supposed that any lactone groups present are opened by this procedure (*10*), but other reactions liberating carboxyl groups may also occur, especially when a large excess of alkali is used (*22*). Another electrolyte often added is calcium acetate (*15*). In this case the buffering action of the salt and the strong affinity of the calcium ions for carboxyl groups cause the ion exchange to take place before titration, although the same buffering action makes the method less reliable when the carboxyl content is low (*32*).

A further method of determining the hydrogen ions is based upon iodometric titration (*17*).

For the determination of carboxyl groups the paper can also be converted into the calcium form, whereupon the calcium can be determined after being exchanged for hydrogen ions (*26*) or by electrodialysis (*25*). As this method is a matter of equilibrium reactions, there are advantages in carrying out this process chromatographically (*20*).

Other methods for carboxyl determinations, involving the use of ions of heavy metals, are known for silver (*27*), copper(II) (*23*), tin(II) (*8*), lead(II) (*8, 23*), and uranyl(II) ion (*4*). Often, use is made of ion exchange with methylene blue (*1, 28*), although this method may involve some difficulties.

Considerable uncertainty existed in most of the previously used methods for carboxyl determination. Although the ion exchange of filter paper is usually ascribed to the presence of carboxyl groups, it was believed that part of the ion exchanging groups might be present in the form of lactone or ester groups, which are easily opened (*9, 10, 16*). Hence, an attempt was made, with the aid of infrared spectroscopy, to determine whether the presence of such groups might be responsible for some error in carboxyl group determinations.

A chromatographic method of estimating the carboxyl content of filter paper has been outlined recently (*24*). A measured quantity of hydrogen or alkaloid ions was used to saturate the available carboxyl groups in a certain area of the paper. After elution the spot was made visible by spraying, and its area was determined; this area should be inversely proportional to the carboxyl content of the paper. Similar investigations had been proceeding in the authors' laboratory for some time with the use of various metal ions to establish their suitability for carboxyl determinations. The best results were obtained with lead ions, which are strongly bound to the carboxyl groups and can be easily detected with hydrogen sulfide. Directions have been worked out for this case.

EXPERIMENTAL

Modification of Filter Paper. Samples of Whatman No. 1 filter paper and cellulose powder were oxidized with nitrogen dioxide gas (*34*) in the absence of air, with a view toward introducing carboxyl groups. The concentration was 0.5 gram of nitrogen dioxide per liter; the ratio nitrogen dioxide to cellulose was 1 to 6 grams; the reaction period was 18 hours. The treated cellulose was thoroughly washed with water and dried. Under these conditions, filter paper is obtained with about 1.5% COOH, determined by titration with calcium acetate (*34*). The presence of lactone groups was investigated by spraying with hydroxylamine and iron chloride (*30*).

Samples of both untreated cellulose and oxidized cellulose were treated with a solution of diazomethane in ether for 18 hours at a temperature below 5° C. The free carboxyl groups are converted into methyl esters by this procedure.

A part (1 gram) of the oxidized cellulose powder was treated with 160 ml. 0.2*M* calcium acetate for 30 minutes, as is customary for the carboxyl group determination (*34*). The calcium salt was then filtered off and dried.

Infrared Spectroscopy. Infrared spectra in the range of 5.5 to 6.5 microns were recorded for slurries of cellulose powder in paraffin oil (about 20 mg. of cellulose, 270-mesh, in 100 mg. of

Table I. Reproducibility of Determinations

Weight of Spots, Mg.[a]			
1.	58.95	9.	58.25
2.	59.30	10.	58.05
3.	57.45	11.	59.40
4.	59.90	12.	57.25
5.	58.10	13.	55.55
6.	56.25	14.	56.50
7.	57.60	15.	58.00
8.	58.05	16.	55.70
Average			57.77 mg.
Standard deviation			2.16%

[a] Obtained by applying 4.5×10^{-4} meq. of lead acetate on a strip of Whatman No. 1.

Table II. Determination of Error in Calculating Weights of Spots

Weight of Spots, Mg.[a]			
1.	40.07	11.	41.30
2.	40.00	12.	40.50
3.	40.50	13.	40.70
4.	40.90	14.	40.10
5.	40.50	15.	41.20
6.	40.30	16.	41.70
7.	40.80	17.	39.48
8.	39.80	18.	41.80
9.	40.85	19.	40.45
10.	40.45		
Average			40.60 mg.
Standard deviation			1.48%

[a] One spot is copied and weighed 19 times.

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paraffin oil). Similar spectra of modified cellulose, galacturonic acid, glucuronic acid lactone, and calcium galacturonate were recorded.

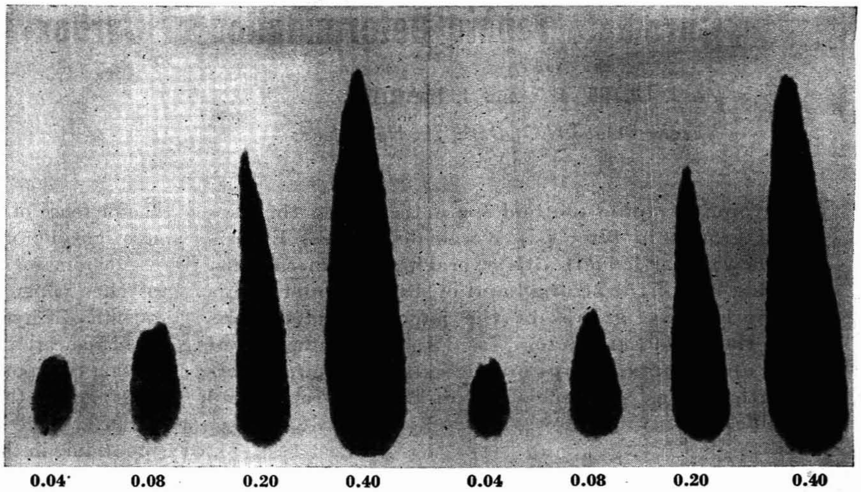

Figure 1. Spots obtained by applications of 10 μl. of lead acetate with concentration of 0.04, 0.08, 0.20, and 0.40*M*

***Right.* Duplicate spots**

Chromatographic Determination of Carboxyl Groups. Procedure. By means of a micropipet 10 μl. of lead salt solution (acetate or nitrate) of known concentration (0.01 to 0.1*M*, depending on the type of paper) is applied on a strip of paper, about 2 cm. from the lower edge. Immediately afterwards the strip is suspended in boiled distilled water (water and strip are kept in an enclosed space) in such a way that the bottom of the strip is just in the water. If necessary, the paper is stretched by means of a glass hook. The water is then allowed to rise to a height of at least 10 cm., which usually takes place within half an hour. The lead spot is made visible by reaction with hydrogen sulfide. In order to measure the area, it is traced on a heavy type of sized paper with the aid of carbon paper. The spot thus copied is cut out and weighed. If the weight per square centimeter of the types of paper is known, the area of the spot and the carboxyl content of the paper can be calculated.

The same procedure has been used with solutions of stannous chloride, copper sulfate, silver nitrate, mercuric acetate, and ferric nitrate. In the case of uranium salts, the spots were made visible by coating them with powdered potassium ferrocyanide, followed by steaming. With methylene blue the spots are visible as such.

RESULTS AND DISCUSSION

If the surface area of the spots is plotted against the number of milliequivalents of lead(II), a straight line is obtained for all types of paper, which almost passes through the datum point (Figures 1 and 2).

With uniform types of paper the reproducibility of various determinations is satisfactory. For Whatman No. 1, for example, the standard deviation from the average was 2.16% (Table I). The error in the determination of the size of the spots is still smaller (Table II). Usually no difference is found for elution periods varying between 20 minutes and 24 hours, but with some types of paper the water rises so slowly that the definite size of the spot is not reached within 20 minutes (Table III).

Table III. Influence of Variation of Elution Periods

Time, Min.	Weight of Spots, Mg.[a]
20	26.6
30	27.8
60	25.8
180	28.5
24 Hours	27.2

[a] Obtained by applying 8.2×10^{-4} meq. lead acetate on Schleicher and Schüll No. 589. For Schleicher and Schüll 1101L an elution period of almost 60 minutes is necessary.

It is known from the literature that the affinity of different cations for carboxyl groups varies. In this connection it is possible to draw up a series (*2, 23*):

$$UO_2^{++} \approx Pb^{++} > Sn^{+r} > Cu^{++} > H^{+} > \text{methylene blue}^{+} > Ba^{++} > Ca^{++} > Ag^{+} > Tl^{+} > K^{+} > Na^{+} > Li^{+} > N(CH_3)_4^{+}$$

The most striking feature is the strong bonding of the hydrogen ion (*13*) and of some multivalent metals of the secondary groups. The lead ions are capable of displacing the hydrogen ions, so that the carboxyl determinations are fairly independent of the pH. Consequently, pretreatment of the paper with acid does not affect the results (Table IV). It may be assumed that in this process all carboxyl groups react with the lead ions. The spot will shift only on the addition of complex-forming ions to the elution agent (*31*). Complex formation also seems to be responsible for the fact that the method cannot be used with mercury(II) and iron(III) ions. Copper(II) and tin(II) ions, on the other hand, produce very clear spots. However, these are larger than the spots obtained in the lead method (Table V); hence it becomes evident that part of the carboxyl groups have not reacted with copper(II) or tin(II) ions.

Uranyl(II) is one of the most firmly bound ions. [The determination of carboxyl groups in carboxymethylcellulose (*6*) is actually carried out at a pH as low as 4.] The chromatographic determinations have shown, however, that with certain types of paper, uranyl ions exhibit abnormal absorption phenomena as a result of which the spots obtained are too small. The use of silver ion leads to complications if reducing groups are present in the paper (*3, 22*). Methylene blue produces very clear spots, but in a weakly acid medium complete ion exchange does not take place, owing to its position in the ionic series.

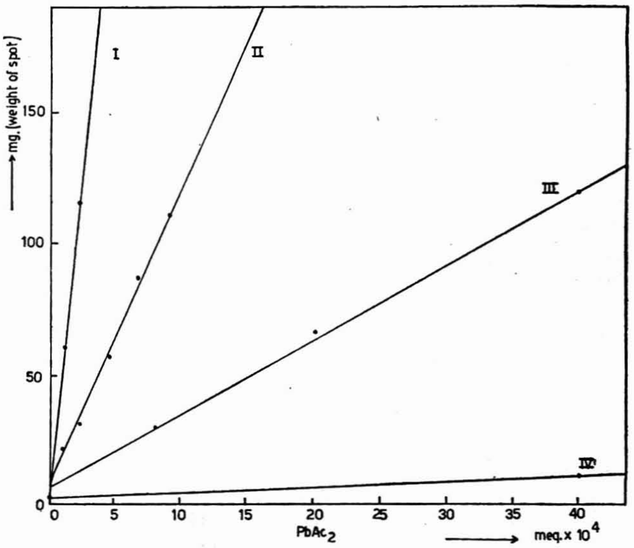

Figure 2. Carboxyl content

I. Methyl ester of Whatman No. 1
II. Whatman No. 1
III. Schleicher and Schüll No. 589
IV. Oxidized Whatman No. 1

Table IV. Influence of Pretreatment of Filter Paper on Carboxyl Determination

(Treated papers washed with dilute HCl and water)

Type of Paper	Lead Acetate, Meq.	Weight of Spot, Mg.	
		Untreated	Treated
Whatman No. 1	9.7 × 10^{-4}	115	111
Schleicher and Schüll No. 1101L	19.4 × 10^{-4}	51	56
Macherey, Nagel No. 613	19.4 × 10^{-4}	32	35

Table V. Determination of Carboxyl Groups with Lead Acetate and Copper Sulfate on Whatman No. 1 Paper

Salt Solution Used, Meq.	Weight of Spot, Mg.	
	Copper sulfate	Lead acetate
1.94 × 10^{-4}	46.7	31.8
3.88 × 10^{-4}	85.0	49.3
5.82 × 10^{-4}	123.0	71.2

The results obtained with the lead method for various types of paper have been summarized in Figure 2 and Table VII. The calcium acetate method usually produces higher values (Table VI). With a view toward ascertaining whether this difference must be ascribed to the presence of easily opening lactone or ester groups, the authors recorded infrared spectra in the range 5.5 to 6.5 microns (Figures 3 and 4).

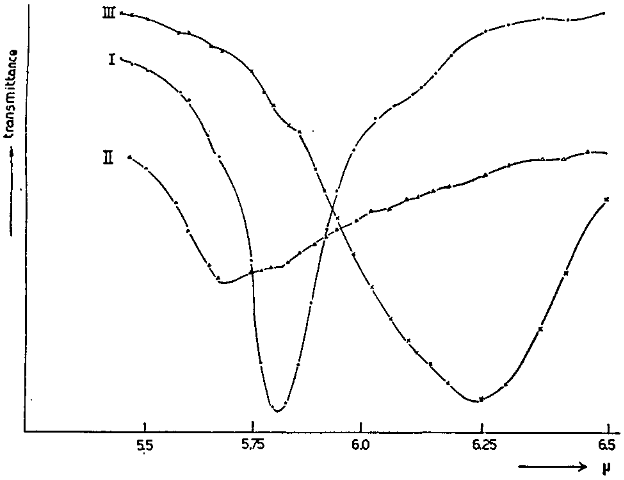

Figure 3. Infrared spectra

I. Galacturonic acid
II. Glucuronic acid lactone
III. Calcium galacturonate

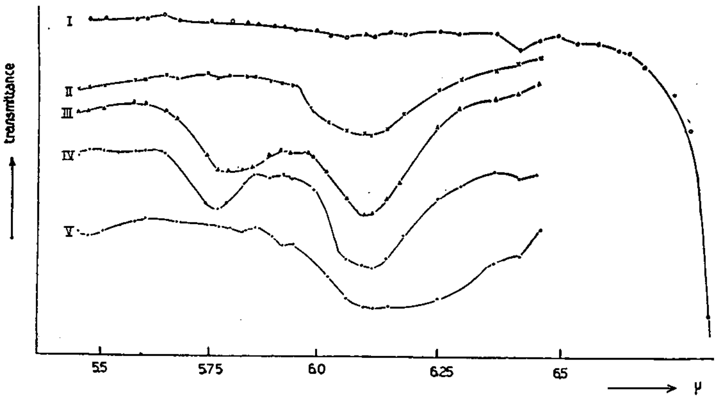

Figure 4. Infrared spectra

I. Paraffin oil
II. Untreated cellulose
III. NO_2-oxidized cellulose
IV. Methyl ester of NO_2-oxidized cellulose
V. Calcium salt of NO_2-oxidized cellulose

In the case of galacturonic acid (Figure 3, I) (see Figure 5 for the formula), a pronounced absorption peak is found at 5.8 microns which is attributed to the stretching frequency of the C=O bond of the carboxyl group. With glucuronic acid lactone (Figure 3, II), the peak occurs at 5.7 microns; with calcium galacturonate, on the other hand (Figure 3, III), the salt form maximum absorption occurs at 6.25 microns.

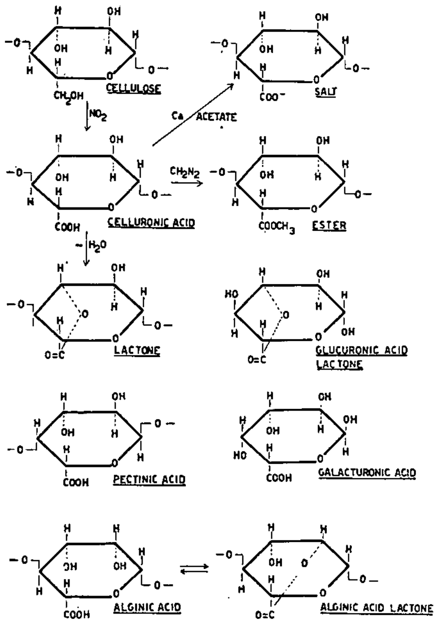

Figure 5. Hexose derivatives, mentioned in text

The spectrum of untreated cellulose (Figure 4, II) exhibits absorption only in the range of 6.1 microns, which is ascribed to a bending frequency of absorbed water (*21*). In the case of oxidation with nitrogen dioxide (Figure 4, III), absorption appears at 5.8 microns, which could indicate the presence of both acid and lactone. In the case of treating the oxidized cellulose with diazomethane (Figure 4, IV), the peak is sharper and lies at 5.75 microns. When oxidized cellulose is titrated according to the calcium acetate method, the infrared spectrum of the calcium salt formed (Figure 4, V) no longer shows any signs of absorption in the range of 5.75 microns. It must be assumed, therefore, that under these conditions the lactone ring is split open. The titration of alginic acid (*16*) is somewhat similar, since in a dry state its carboxyl groups are nearly all in the lactone form (*11*).

During the titration of glucuronic acid lactone in the presence of calcium acetate considerable drift was found at the end point. This indicates that the lactone ring opens, especially in a weakly alkaline medium.

In applying the lead method to the methyl esters of Whatman No. 1 and nitrogen dioxide–oxidized filter paper, it was invariably found that part of the carboxyl groups were still reacting (Figure 2 and Table VII). In particular with esterified "celluronic acid," the wide variation in spot sizes is indicative of partial splitting of ester during the chromatography. Whatman No. 1 gave no visible reaction when sprayed with hydroxylamine and iron(III) chloride;

Table VI. Results Obtained with Calcium Acetate and Lead Method

Type of Paper	Carboxyl Content, Meq./Gram	
	Calcium acetate	Lead acetate
Schleicher and Schüll No. 1101L	0.08	0.046
Macherey, Nagel No. 613	0.10	0.064
Oxidized Whatman No. 1	0.65	0.420

Table VII. Carboxyl Content of Some Filter Papers

Type of Paper	Carboxyl Content, Meq./Gram
Whatman No. 1	0.008
Methylester of Whatman No. 1	0.002
Oxidized Whatman No. 1	0.420
Methylester of oxidized Whatman No. 1	0.017
Whatman No. 4	0.007
Munktell No. 20	0.007
Schleicher and Schüll No. 589	0.035
Schleicher and Schüll No. 1101L	0.046
Macherey, Nagel No. 613	0.064

nitrogen dioxide–oxidized paper showed a faint color and the methyl esters of these papers colored strongly. It is evident that oxidized cellulose contains a certain quantity of lactone groups which are readily split open. Such groups can also occur in native cellulose; therefore, no conclusions should be drawn regarding the acid value of the ionizable groups by extrapolation of titration curves (*33*).

In titrations with an excess of alkali the splitting of lactone is probably not the principal reaction involving the liberation of extra carboxyl groups. Carbonyl groups can, in an alkaline medium, be subject to conversions resulting in the formation of carboxyl groups. Furthermore, cellulose is very sensitive to oxidation at a high pH value.

Apart from difficulties arising at very high lactone concentrations, the chromatographic determination of carboxyl groups carried out by means of lead ions seems to be a rapid and reliable method for determining the carboxyl content of filter paper.

ACKNOWLEDGMENT

The authors express their sincere thanks to G. J. Schuringa for his encouragement, to J. R. H. van Nouhuys, director of the Vezelinstituut T.N.O. (Fiber Research Institute T.N.O.), for his permission to publish the results of this investigation, and to R. C. Blume and G. N. Milford for discussing and correcting the manuscript.

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Received for review April 28, 1954. Accepted October 25, 1954.

Rapid Determination of Water in Silicate Rocks

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A rapid and simple method for the determination of total water in silicate rocks has been developed by modifying the Penfield procedure. In this method, the time required for a single determination has been reduced to less than 10 minutes. Comparison of the data obtained by this modification and the Penfield method indicates the same degree of accuracy.

THE value obtained for total water in a rock analysis includes water of crystallization, water held uncombined in the grains or on their surfaces, and water formed, as the result of heating, from hydrogen or hydroxyl groups present in regular atomic arrangement in molecular or crystal structure.

The determination of water in rocks invariably involves initial ignition of the powdered sample, either by itself or with a flux, and subsequent measurement of the expelled water. The expelled water can be measured in several ways. It can be absorbed in a desiccant in a preweighed tube (*1*), condensed and determined by measurement of volume (*3*), or measured as in the method of Penfield (*4*), which is more widely used than any other method. In the Penfield method the upper part of the glass tube, containing the condensed water expelled from the sample powder, is separated by fusion from the lower part of the glass tube, containing the powder. The part containing the water must be allowed to reach thermal and moisture equilibrium with the laboratory air before weighing. A second time-consuming heating and equilibration step is necessary in getting the final or "dry tube" weight to subtract from the initial weight to obtain the amount of water in the sample. Because of these time-consuming steps, the

Penfield method, though it takes less time and is simpler than other methods, usually requires 30 to 40 minutes for a single determination.

A value for water can also be obtained by determination of loss on ignition. Values so obtained vary in accuracy from good to poor depending upon the composition of the samples.

The U. S. Geological Survey has need for analyses of many rock samples and has described rapid methods for the determination of the major constituents of silicate rocks (*5*). In this described analysis an "ignition loss" determination is used in lieu of a water determination. For samples in which carbonate and ferrous iron concentrations are low, values based on ignition loss determination are entirely adequate. When the samples contain appreciable carbonate or appreciable ferrous iron or both, direct determination of water is desirable.

The method described—a modification of the Penfield procedure—requires less than 10 minutes per sample and was developed to meet the need for a rapid method for the determination. It consists of expelling the water from the sample in a test tube, absorbing the water on a preweighed strip of filter paper placed inside the tube, and subsequently reweighing the paper. The difference in the weight of the paper before and after absorption of the water is then used, after correction for water lost in handling, to calculate per cent water in the sample.

APPARATUS AND REAGENTS

Test tube for ignition, 18 × 150 mm., borosilicate glass.

Test tube for weighing paper, 18 × 65 mm.

Rubber stopper for ignition tubes, one-hole, No. 1.

Filter paper strips, 2 inches square cut from any grade of paper except hardened types.

Funnel for transfer of sample to bottom of ignition tube.

Analytical balance.

Finger stalls or rubber gloves for covering the tips of the fingers of one hand.

Support to hold the test tube during ignition.

Burner, Fisher type.

Anhydrous sodium tungstate for use with samples containing sulfur. Fuse several hundred grams of reagent sodium tungstate dihydrate ($Na_2WO_4.2H_2O$). Cool and grind to a fine powder and store in a bottle with a tight sealing cap.

PROCEDURE

Transfer 1.000 gram of sample powder to a dry 18 × 150-mm. borosilicate glass test tube by means of a funnel. (For samples containing an appreciable amount of sulfur it is advisable to mix 3 grams of anhydrous sodium tungstate with the sample powder prior to ignition to prevent high results.) Cover the fingers of the hand with finger stalls or a rubber glove. Roll a piece of filter paper (2 inches square) into a cylinder and slip it into the 18 × 65-mm. weighing tube; stopper the tube with a solid stopper and weigh. (To avoid absorption of moisture from the fingers, the paper should be handled only with covered fingers.) Quickly transfer the paper cylinder from the weighing tube to the upper part of the 18 × 150-mm. test tube, which contains the sample powder and stopper with a one-hole stopper. Next, place a piece of wet paper (2 × 3 inches) around the outside of the upper portion of the ignition tube to cool the tube. Then place the tube in a horizontal position in a holder at a height that will allow the tube to get maximum heat from a Fisher burner (Figure 1). Heat the closed end of the tube gently at first, then at full heat of the burner for 5 minutes. The flame should then be played along the tube below the filter paper for 1 or 2 seconds to drive the water into the upper part of the tube. Allow the tube to cool for at least a minute. Remove the stopper, and, with the aid of a rod or narrow spatula, quickly wipe the walls of the tube surrounding the paper by gently pressing and rotating the filter-paper cylinder for one full rotation. With the fingers covered quickly transfer the paper from the test tube to the weighing tube, stopper, and weigh the tube. The increase in weight represents the water caught by the filter-paper strip.

A small amount of the expelled water is not condensed and caught by the filter-paper strip. Part is driven out of the tube and part remains as unabsorbed vapor in the tube. To take care of these losses a simple correction is applied, based on an empirically determined rule. If the amount of water absorbed on the filter paper is 20 mg. or less, increase the value obtained by 10%. If the absorbed water is over 20 mg., add 2 mg. to the weight of water absorbed. The result obtained after the correction is applied is equal to the weight of water in the sample.

Table I. Relationship between Water in Sample, Absorbed and Computed Waters

Water, Mg.			Error, Mg.	
In sample	Absorbed	Computed[a]	Experimental	After correction
4.0	3.2	3.5	−0.8	−0.5
5.0	4.6	5.0	−0.4	0.0
8.0	7.4	8.1	−0.6	+0.1
10.0	9.0	9.9	−1.0	−0.1
13.4	12.2	13.4	−1.2	0.0
20.0	18.5	20.5	−1.5	+0.5
26.8	25.5	27.5	−1.3	+0.7
40.2	39.0	41.0	−1.2	+0.8
50.0	48.0	50.0	−2.0	0.0
67.0	65.9	67.9	−1.1	+0.9
80.0	78.2	80.2	−1.8	+0.2
100.0	98.2	100.2	−1.8	+0.2
134.2	132.1	134.1	−2.1	−0.1
150.0	147.2	149.2	−2.8	−0.8
167.5	165.2	167.2	−2.3	−0.3

[a] Correction rule applied. For range 0.0 to 20.0 mg. of absorbed water add 10% of the amount obtained; above this value add 2.0 mg. to the amount obtained.

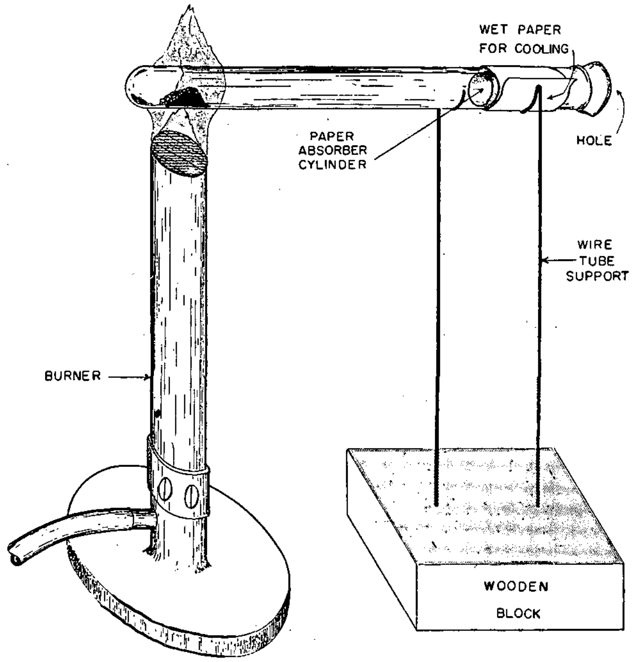

Figure 1. Apparatus for determination of water

EXPERIMENTAL DATA

Effect of Handling Filter Paper with Bare Fingers. A 2 × 2-inch piece of filter paper, an 18 × 65-mm. weighing tube, and a stopper were placed on the pan of the balance with the bare fingers and weighed. With the bare fingers the paper from the balance pan was rolled into a cylinder and inserted into the weighing tube. The tube was stoppered and weighed. An increase in weight of about 3 mg. was observed. The experiment was repeated but with the fingers covered by rubber finger covers. No change in weight was observed. Each experiment was repeated three times, with no change in results. These experiments indicate the desirability of avoiding contact of the bare fingers with the filter-paper absorber.

Correction for Unabsorbed Water. A small part of the water expelled from the rock samples fails to be absorbed on the filter-paper strip. A part of the unabsorbed water is driven out of the tube as a result of the expansion of the air in the tube when the tube is heated. An additional small amount remains unabsorbed as vapor in the tube.

A series of tests was made to relate the amount of water absorbed to the amount of water actually present in the samples in the concentration range anticipated for rock samples. Different amounts of National Bureau of Standards Standard Sample No. 97 (clay) were carried through the procedure. In Table I the amounts of water absorbed are tabulated against the amounts of water contained in the several sample weights. The results obtained by two analysts have been used in the preparation of the table. In each case, the amount of water absorbed is a little less than that originally in the sample portions. Inspection of the data shows that a simple rule can be used to correct for the deficiency in the amount of water absorbed. On samples for which the water absorbed is 20 mg. or less the value should be increased by 10%, and on samples for which the water absorbed is 20 to 160 mg. the value should be increased by 2 mg. Table I also shows the result of applying this rule to the set of data.

Table II. Precision

Sample	9[a]	10[a]	11[b]	12[b]
Water, %	0.23 0.28 0.28 0.29 0.29 0.30	1.9 1.9 1.9 1.9 1.9 1.9	7.9 7.7 7.9 7.8 7.8 7.7	13.2 13.4 13.4 13.2 13.2 13.2
Average	0.28	1.9	7.8	13.3

[a] Determined by Leonard Shapiro.
[b] Determined by Harry F. Phillips, U. S. Geological Survey.

RESULTS

Precision of the method has been tested by running samples six times at each of four concentration levels. The results are shown in Table II. Results obtained by the rapid method for total water in eight rock samples by two analysts are compared in Table III with results obtained by a third analyst who used the conventional Penfield procedure. The values are in close agreement.

Table III. Comparison of Results by Rapid and Penfield Methods

Sample	Water, % Rapid A[a]	Water, % Rapid B[b]	Water, % Penfield C[c]
W-1[d]	0.61	0.59	0.59
G-1[d]	0.30	0.19	0.34
1	0.58	0.50	0.54
2	0.40	0.36	0.35
3	1.0	0.94	1.0
4	2.1	2.2	2.1
5	2.3	2.2	2.4
6	2.2	2.1	2.3
7	7.8	7.7	7.8
8	13.4	13.2	13.4

[a] Determinations by Leonard Shapiro.
[b] Determinations by Harry F. Phillips, U. S. Geological Survey.
[c] Determinations by Paul W. Scott, U. S. Geological Survey.
[d] Two carefully prepared rocks for collaborative study (*2*).

A collaborative study of silicate rock analysis (*2*) reports the results of water determinations by the Penfield method and some of its modifications. The reported values varied by 0.3 to 0.4% at the 0.5% level of water. The rapid method gives values well within the range indicated by this collaborative study.

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Received for review October 23, 1954. Accepted December 16, 1954. Publication authorized by the director, U. S. Geological Survey.

Polarographic Determination of Sulfite

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A method was needed for determining sulfite in the presence of other reduced compounds, in order to study the transformation of sulfur compounds in biological sewage treatment. The use of the polarograph is satisfactory for determining sulfite in concentrations of 1 to 250 p.p.m. as sulfur. The method involves the deaeration of the sample in neutral or alkaline conditions, acidification of the sample to convert all the sulfite to sulfur dioxide, determination of the height of the anodic wave produced by the sulfur dioxide, desorption of the sulfur dioxide, and determination of the height of the wave at the same applied potential in the absence of the sulfur dioxide. The difference between the two determinations is directly proportional to the concentration of sulfite originally present.

An ACCURATE method for the determination of sulfite in the range of 0 to 250 p.p.m. was needed for the study of sulfur transformations occurring in waste treatment processes.

The two methods more frequently used for determining sulfite in low concentrations, oxidation and subsequent determination as sulfate (*4*) and oxidation with iodine (*5*), are subject to interferences from other inorganic sulfur compounds and other oxidizing or reducing compounds, which occur in sewage and industrial wastes.

Therefore, a method had to be found which was sufficiently sensitive and accurate to measure low concentrations of sulfite in highly heterogeneous mixtures, such as wastes. Kolthoff and Miller (*2*) showed that sulfite in $0.1N$ nitric acid produced a polarographic wave in pure solutions of sodium sulfite having concentrations of approximately $5 \times 10^{-4}M$ (15 p.p.m. as sulfur). This indicated that the polarographic method was sufficiently sensitive; however, it had to be proved that this method would give accurate results when used to analyze waste samples of a highly complex nature.

APPARATUS

The apparatus used was the Fisher Elecdropode. The scale of the galvanometer was calibrated and it was found that each division of the scale at the 1X sensitivity was equivalent to 0.0186 μa. The temperature of the samples was maintained at 25° C. by immersing the polarographic cell in a constant temperature water bath. Water-pumped nitrogen was used for deaeration of the samples.

EXPERIMENTAL

Effect of pH. The nitric acid supporting electrolyte used by Kolthoff and Miller was tried. However, the deoxygenation of the sample prior to polarographic analysis resulted in the desorption of the sulfite, present as sulfur dioxide in such acid conditions. Although the loss of sulfur dioxide from the sample initially appeared to be detrimental, it later served as the basis for the method of sulfite determination described in this paper.

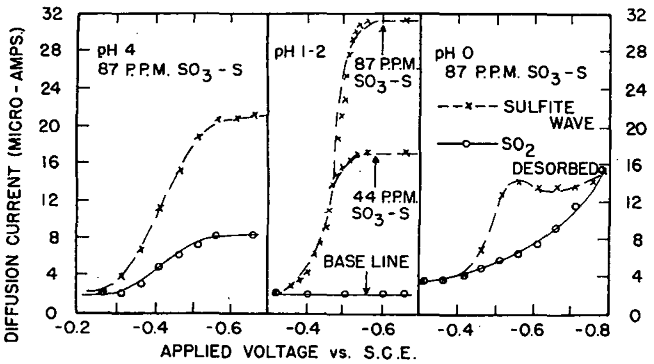

Figure 1. Effect of pH on polarographic sulfite wave

It was found that neutral or alkaline sulfite solutions could be deoxygenated with nitrogen without the loss of sulfur dioxide. When this was followed by acidification with nitric acid, the sulfur dioxide formed could be quantitatively removed by further scrubbing with nitrogen. The difference between the diffusion current immediately after acidification and after removal of the sulfur dioxide was directly proportional to the sulfite concentration. That the sulfur dioxide was completely removed was shown by the return of the diffusion current to the same value as that of the base electrolyte alone.

For the conversion of sulfite to sulfur dioxide and its subsequent quantitative desorption, a pH of 1 to 2 was needed. Figure 1 shows the effects of pH upon the determination. Above pH 2, the sulfite was not quantitatively converted to sulfur dioxide, as shown by the reduced diffusion currents accompanying the higher pH values. Also, the sulfur dioxide was not quantitatively removed by scrubbing with nitrogen, as shown by the fact that the diffusion current was greater than the residual current of the base electrolyte. Between pH 1 and 2 the diffusion current was directly proportional to the sulfite concentration. As the pH was decreased below 1, the diffusion current continually diminished for solutions of the same sulfite concentration. This may be due to the evolution of nitrogen dioxide gas, produced by the reaction between mercury and nitric acid, which partially desorbs the sulfur dioxide. The evolution of nitrogen dioxide was not observed at pH 1 or above. In addition to the polarographic wave produced by the reduction of sulfur dioxide another wave of different character appeared within the same voltage range, thereby complicating the sulfite determination below pH 1. This wave was very similar to the one produced by 1*N* nitric acid alone. Within the pH range of 1 to 2, the current produced was maximum and directly proportional to the sulfite concentration. Therefore, this pH range was used for all the polarographic sulfite determinations.

Prevention of Sulfite Oxidation. Pure solutions of sulfite at pH 7 or greater are rapidly oxidized in the presence of air. However, Mangan (*3*) shows that the presence of 5% glycerol or sucrose greatly reduced the rate of oxidation. Neither glycerol nor sucrose interfered with the sulfite determination, and they were responsible for more reproducible results.

Nature of the Polarographic Curve. A typical polarogram for the reduction of sulfur dioxide in the pH range of 1 to 2 is shown in Figure 1. The diffusion current reached a maximum at −0.60 volt *vs.* the saturated calomel electrode (S.C.E.). The potentials recorded in this figure have not been corrected for the *IR* drop of the solution. The resistance, *R*, of the solution was found to be 950 ohms. Each part per million of sulfite sulfur caused a galvanometer deflection of 18 divisions, which is equivalent to 0.335 μa. The applied potential of −0.6 volt was chosen as standard for the determination.

Interferences. Measurement of the sulfite concentration as the difference between the diffusion current after acidification and after the desorption of the sulfur dioxide eliminated most interferences. In addition to sulfite, there may be other substances present in waste samples which are reduced at a potential of −0.60 volt *vs.* S.C.E. or less. Those which are not desorbed with the sulfite will produce the same increase in the diffusion current both before and after the removal of the sulfur dioxide from the acidified sample and will produce no error. Those which are desorbed with the sulfur dioxide by nitrogen will produce a positive error.

Sulfide in any concentration interfered with the sulfite determination (Table I) due to an anodic current produced by the sulfide in the region of −0.6 volt (*1*). The effect of sulfide may be eliminated by precipitating it with zinc acetate or by the method of Mangan (*3*). Thiosulfate also interfered with the sulfite determination. While not producing a wave of its own, the thiosulfate in the presence of sulfite produced a rapidly increasing diffusion current with any small increase in the applied potential, so that a limiting diffusion current was never reached. Thiosulfate may be removed by the differential solubility of the lead salts of sulfite and thiosulfate as described by Mangan (*3*).

METHOD

Standard Curve. To prepare a standard curve of sulfite concentration *vs.* diffusion current, a solution of known sulfite concentration is used; this is standardized by adding a known volume of iodine and back-titrating the excess iodine with standard sodium thiosulfate, using starch as an indicator (*5*). Sufficient glycerol is added to produce a 5% concentration in the standard sulfite solution. Using this standardized sulfite solution, the height of the polarographic wave is determined as described below. Knowing the galvanometer deflection for a known concentration of sulfite, the deflection for each part per million is determined. This relationship is linear for the concentration of 1 to 250 p.p m. of sulfite sulfur.

Preparation of Sample. To the sample is added sufficient glycerol to produce a 5% concentration. Particulate matter is removed by centrifugation. Any sulfide present is precipitated by the addition of zinc acetate and removed by centrifugation. Thiosulfate present is separated from the sulfite by the method of Mangan (*3*). The pH of the sample is adjusted to 7 or higher.

Analysis. A 15-ml. portion of the prepared sample containing 1 to 200 p.p.m. of sulfite sulfur is placed in the polarographic cell. The oxygen is removed by scrubbing with nitrogen for 5 minutes. The nitrogen bubbler is then removed, and the sample is acidified with a few drops of concentrated nitric acid to lower the pH to 1 to 2. Five drops of acid are usually sufficient to adjust the pH of a 15-ml. sample, but for exceedingly alkaline or well-buffered samples, the amount of acid to be added should be determined on a separate portion of the prepared sample. The mixture is stirred for a few seconds with a small stirring rod. The bubbler is placed so as to maintain a nitrogen atmosphere over the sample. With an applied potential of −0.6 volt *vs.* S.C.E., the diffusion current is noted immediately. Nitrogen is bubbled through the solution for 5 minutes to remove the sulfur dioxide. Removing the bubbler and again maintaining the nitrogen atmosphere over the sample, the diffusion current is

Table I. Effect of Sulfide and Thiosulfate on Polarographic Determination of Sulfite

(P.p.m. as sulfur)

Calcd. SO_3 Concn.	Sulfide Concn.	Detd. SO_3 Concn.	Error, %
56	22	61	+5
62	0.8	58	−4
84.0	ca. 200	66.8	−17.2
82.4	ca. 200	74.4	−8.0
82.4[a]	ca. 200	83.1	+0.7
82.4[a]	ca. 200	84.6	+2.2
25.5	247	24.0	−1.5
25.5	247	25.5	0
15	346	13	−2
12.5	371	18	+5.5
12.5	371	14.5	+2.0
12.5	371	13	+0.5
5	450	14	+9
25	352	11	−14
23	391	30	+7
33	78	35	+2

[a] Excess zinc acetate added.

Table II. Polarographic Determination of Sulfite in Various Solutions

(P.p.m. as sulfur)

Solution	Calcd. SO_3 Concn.	Detd. SO_3 Concn.	Error	Error, %
Dist. water	15.2	16.1	+0.9	5.9
	3.8	3.9	+0.1	2.6
	7.6	6.9	−0.7	9.2
	11.4	11.7	+0.3	2.6
	15.2	15.3	+0.1	0.7
	31.0	30.0	−1.0	3.2
	225	222	−3.0	1.3
Sewage	31.0	31.7	+0.7	2.3
Sludge liquor	29.0	29.4	+0.4	1.4
	29.0	26.7	−2.3	7.9
	22.5	21.7	−0.8	3.6
	45.0	47.8	+2.8	6.2
Average			±1.2	3.8

noted at the same applied potential. The difference between the two galvanometer readings is directly proportional to the sulfite concentration in the sample. This proportionality has been determined previously above.

DISCUSSION

The accuracy of the polarographic method is shown (Table II) by the recovery of sulfite added to distilled water, sewage, and sludge liquor from which the sulfite, thiosulfate, and sulfide were removed (*3*). The method is sensitive to at least 1 p.p.m. of sulfite sulfur. The diffusion current produced by this concentration corresponds to 18 divisions (0.335 μa.) on the scale of the galvanometer used. The method is not recommended for use with samples containing more than about 200 p.p.m. of sulfite sulfur because of the longer time required for the desorption of the sulfur dioxide at higher concentrations. Of all the substances present in sewage and sludge liquor, only sulfide and thiosulfate were found to interfere. When these were removed, the average error of the method was reduced to about 4%. The removal of sulfide and thiosulfate from the sample by the method of Mangan (*3*) required about 2 hours. Centrifugation consumed the major portion of this time. Several samples may be treated simultaneously, thereby reducing the time per sample. In most wastes, thiosulfate and sulfide are seldom found in the presence of sulfite. The conditions under which these compounds occurred together were artificially created in order to study the transformations of these sulfur compounds during sewage treatment. After the preparation of the sample, to remove these interfering compounds, the polarographic determination required only 15 minutes.

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RECEIVED for review June 1, 1954. Accepted November 13, 1954. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey Department of Sanitation, New Brunswick.

Spectrophotometric Determination of Aliphatic Sulfides

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A procedure for spectrophotometric determination of aliphatic sulfides has been modified to improve its accuracy and reproducibility.

IN A previous paper (*1*) a procedure was given for the determination of aliphatic sulfides, utilizing the intense ultraviolet absorption spectrum of the complex formed between these sulfides and molecular iodine. Certain modifications have been made to improve the accuracy and reproducibility of the method and an error in the original paper has been noted.

The decrease in absorbance of the iodine-sulfide complex with time while in the cell compartment of the Beckman DU quartz spectrophotometer was thought to be due to the fact that the sample was in the dark; however, it was caused simply by a shift in the equilibrium brought about by the higher temperature of the cell compartment. The thermal effect noted on the Beckman spectrophotometer also occurs with the Cary ultraviolet spectrophotometer (Model 11). The equilibrium involved is

$$R'SR + I_2 \rightleftharpoons R'SR.I_2 \qquad (1)$$

where R'SR is an aliphatic sulfide, I_2 is molecular iodine, and $R'SR.I_2$ is the complex.

This reaction has been found to be temperature-sensitive, lower temperatures favoring the formation of the complex. As a result, it is necessary to perform the analysis under controlled temperature conditions or apply a temperature correction. The temperature coefficient of the "apparent" absorptivity of the complex is approximately −13 absorptivity units per degree Fahrenheit at 79° F., and absorptivity is 372 liters per gram cm. (at thermal equilibrium in the cell compartment). The term "apparent" is employed because the absorption due to the complex has been correlated with the initial sulfide sulfur concentration and this relationship is significantly affected by the temperature.

The iodine concentration specified in the original work was later found to be far from optimum. Study of the iodine-sulfide complex has shown that the complex is a 1 to 1 combination of iodine with the sulfide, as indicated above. The equilibrium constant for this reaction is

$$K = \frac{[R'SR.I_2]}{([R'SR]_i - [R'SR.I_2])([I_2]_i - [R'SR.I_2])} \qquad (2)$$

where *i* indicates the initial concentrations. Calculations based on the iodine and sulfide concentrations specified in the analytical procedure (*1*) show that the two species must be present in very close to a 1 to 1 mole ratio, and as a consequence, the complex concentration is very sensitive to slight changes in the concentration of either of the partners making up the complex. This is undesirable, as it is known that aromatic and olefinic hydrocarbons tie up some iodine and consequently the iodine concentration is not constant, as was previously assumed. However, if the iodine concentration is made large with respect to the sulfide concentration, the factor $([I_2]_i - [R'SR.I_2])$ in Equation 2 is essentially constant and the absorbance due to the complex is significantly affected only by changes in $[R'SR]_i$. Another important advantage results when $[I_2]_i >> [R'SR]_i$. The equilibrium constant can then be written, to a good approximation, as

$$K = \frac{[R'SR.I_2]}{[I_2]_i([R'SR]_i - [R'SR.I_2])} \qquad (3)$$

and

$$[R'SR]_i = \left(\frac{1}{[I_2]_i K} + 1\right) [R'SR.I_2]$$

Therefore, the initial sulfide concentration is linearly related to the complex concentration and a Beer's law equation can be used to calculate the sulfide concentration from the measured absorbance due to the complex. The deviation in the apparent absorptivity for the condition, $[I_2]_i \cong [R'SR]$ is 17% over an absorbance range of 0.5 to 2.0. With the proposed changes in $[I_2]_i$ and $[R'SR]_i$ the deviation is reduced to 4%.

The procedure has been modified to provide a large excess of iodine over sulfides by taking 1 ml. of the appropriate dilute sample and making up to 10 ml. with the stock iodine solution. In this manner, the iodine concentration is increased 9-fold, while the sample concentration is reduced by a factor of 1/9. Under these conditions the absorptivity for sulfide sulfur is 372 liters per gram-cm. (on the Cary instrument) for a cell compartment temperature of 79° F. The absorptivity is adjusted for actual cell compartment temperature using the temperature coefficient of −13 absorptivity units per degree Fahrenheit increase. Thermal equilibrium is generally approached closely in about 10 minutes. The use of a lower sample concentration has the added advantage of permitting the examination of more highly absorbing samples than would otherwise be possible. As a matter of fact, it has been possible to analyze stocks such as heavy gas oils, which generally have considerable absorption near 310 mμ. The sulfide sulfur contents of a number of heavy virgin gas oils, as determined by this method, are shown in Table I, along with the total sulfur content determined by the Dietert method. A significant proportion of the sulfur in these gas oils is of the sulfide type. This sulfide sulfur may be in acyclic or cyclic saturated systems or in side chains attached to aromatic nuclei. It is definitely not directly connected to an aromatic ring (*1*).

A further modification of the method is employed when it is desired to know the sulfide sulfur content of samples whose actual sulfide content is extremely low. In cases of this sort, considerably improved accuracy may be obtained by treating a portion of the sample with solid mercurous nitrate to remove sulfides. This is followed by a wash with 10% aqueous mercuric nitrate solution to assure complete removal, a water wash, and filter-paper drying. The treated sample may then be employed as a blank by adding iodine to the treated sample in the manner in which it is added to the untreated sample. Comparing the two solutions directly on the Cary spectrophotometer yields a delta spectrum which is due only to the sulfide sulfur complex (after a slight correction for differences in cell absorbance). This procedure is more or less restricted to the gasoline boiling range. Attempts to remove sulfides from stocks of gas oil boiling range showed that the mercurous nitrate–sulfide complex, although actually formed, was oil-soluble. Attempts to remove the complex by water washing resulted in destruction of the complex and regeneration of the original sulfides.

Table I. Aliphatic Sulfide Sulfur Content of Virgin Gas Oils[a]

Source of Gas Oil	Sulfur, Weight %	
	Sulfide[b]	Total[c]
West Texas	0.46	2.15
Panhandle	0.25	0.43
Tomball	0.07	0.17
Heavy coastal	0.16	0.27
Hawkins	0.56	2.41
West Texas	0.61	2.05

[a] Nominal 700–1000° F. equivalent atmospheric boiling range.
[b] Determined by revised iodine-complex method.
[c] Determined by Dietert method.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the management of the Humble Oil and Refining Co., in whose laboratories this work was conducted, for permission to publish this paper.

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Received for review July 29, 1954. Accepted November 26, 1954.

Determination of Acetovanillone in Oxidized Alkaline Cleaved Sulfite Liquor by Paper Chromatography

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A rapid method of analysis for acetovanillone in oxidized alkaline cleaved waste sulfite liquor by paper chromatography has been developed. An accuracy within ±5% is indicated.

THE oxidation of waste sulfite liquor in strong caustic solution at elevated temperatures produces vanillin, vanillic acid, acetovanillone, guaiacol, and many other substances with a guaiacyl nucleus (*2*).

To study the formation of cleavage products from waste sulfite liquor, rapid analytical methods are desirable. Stone and Blundell (*3*) have devised a paper chromatographic technique for determining vanillin directly in oxidized alkaline cleavage hardwood liquors.

In this laboratory, experiments on oxidized waste sulfite liquor from gymnosperm lignin showed that it was not possible to separate vanillin from acetovanillone with any of the solvent systems used or modifications of them (*3*). In an analysis for vanillin, the failure to separate vanillin from acetovanillone is of little importance. The vanillin content in a solution of vanillin and acetovanillone is calculated readily from ultraviolet absorbance using a suitable pair of wave lengths. However, where acetovanillone contributes little to the total absorbance, the calculation of the acetovanillone content by this method results in differences of large numbers and gross inaccuracies.

Newcombe and Reid (*1*) reported a method of separation of vanillin from acetovanillone by pretreatment of the paper with sodium bisulfite but gave nothing quantitative nor any applications.

A paper chromatographic method for determining the acetovanillone content of oxidized alkaline cleaved waste sulfite liquor has been developed through which, by the action of the developing solvent, vanillin, acetovanillone, *p*-hydroxybenzaldehyde, and vanillil are separated from the other organics but not completely from one another.

The action of the developing solvent is therefore supplemented by the application, just above the sample, of a bisulfite "block" which retards the vanillin and *p*-hydroxybenzaldehyde but not the acetovanillone or vanillil. The acetovanillone content is then determined in the presence of the vanillil as described below.

EXPERIMENTAL

To a strip of Whatman No. 1 filter paper, 2.5 inches wide and 15.5 inches long and at a position 2 inches from one end, approximately 60 mg. of liquor is added from a micropipet designed for the purpose. The exact amount is determined by weighing the pipet before and after delivery. (The pipet consists of a reservoir of about 1-ml. capacity, with a fine capillary tubing on either end about 2.5 inches long. One end is ground to a point to serve as delivery tip.) The sample is acidified by holding the paper over boiling glacial acetic acid for a few moments. Then approximately 0.1 ml. of a warm solution of 41% sodium metabisulfite is added in a band about 0.5 inch wide just above the sample. Too low a concentration of bisulfite will not effectively stop the vanillin; too high a concentration picks up water and provides a barrier which retards the progress of the developing solvent. The bisulfite band is allowed to air dry or is dried over a hot plate and the paper is then placed over water in a closed container for about 0.5 hour in order to bring the paper to equilibrium with water vapor at room temperature. When completely humidified, the lower end is dipped into *n*-butyl ether (*A* cut, boiling point 141–143° C., from Carbide and Carbon's technical grade, is satisfactory) and the chromatograph allowed to develop 2 to 2.5 hours.

After development the paper is air dried, held briefly over ammonia vapors, and the position of the acetovanillone detected by ultraviolet light. The acetovanillone band is cut from the paper in small squares and dropped into a beaker containing 10 ml. of a phosphate buffer, 0.02635*M* disodium phosphate and 0.00575*M* monosodium phosphate, at pH 7.3. The paper is leached for about 1 hour with one or two very gentle swirls during the leaching period. (The swirling must be very gentle, for too much or too vigorous swirling breaks down the paper, resulting in a rather high and variable paper blank. In a well-executed experiment the paper blanks will not exceed a reading of 0.010 on the absorbance scale of the Beckman spectrophotometer.) The absorbance of the solution is then measured with a Beckman Model DU spectrophotometer at 4000, 3600, 3375, 3200, and 2750 A. with a buffer solution in the reference cell obtained by leaching a blank piece of paper carried through a procedure identical with the sample. (Several blanks are checked with one another and an average one is selected.)

The concentration of the acetovanillone in the buffer can be calculated from the following equation:

$$\text{Acetovanillone concn.} = \frac{D'' - D'\,(kv''/kv')}{ka'' - ka'\,(kv''/kv')}$$

where D'' and D' refer to the absorbance at any two wave lengths, ka'' and ka' the corresponding extinction coefficients for acetovanillone, and (kv''/kv') the ratio of the extinction coefficients of vanillil at the same pair of wave lengths.

In routine practice, the absorbance of the buffer solution at 4000 A. is subtracted from the absorbance at 3375 and the difference divided by the extinction coefficient of acetovanillone at 3375 A., was found to be 44.4 sq. cm. per mg. The absorbances of vanillil at 3375 and 4000 A. are the same, whereas the absorbance of acetovanillone in pH 7.3 buffer is zero at 4000 A.

Chromatographing of pure acetovanillone at a concentration level of 0.05 to 0.1% showed a recovery of 90 to 91%. The final results should be corrected accordingly.

It is not essential to know or to identify the substance for which correction is made in the analysis for acetovanillone. However, the ratio of the absorbances of the interfering material at a selected pair of wave lengths is required.

The spectrum of the interfering material was determined in two ways. First, several strips of paper with chromatographs developed were cut to include only the interfering material. This was possible because the interfering material has a slightly higher R_f value than acetovanillone. (The acetovanillone was located by ultraviolet light and by spraying with 2,4-dinitrophenylhydrazine.)

The cuttings were extracted with ether. The ether was filtered, dried with anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was taken up in buffer, pH 7.3, and its spectrum determined. Second, 12 strips of paper with developed chromatographs were cut to include both acetovanillone and the yellow interfering material, and the combined cuttings leached with 25 ml. of distilled water. The solution was chromatographed on a micro ion exchange column consisting of a fine capillary about 3 inches high packed with Duolite A-2 (160 to 200 mesh) in the hydroxyl form. The binding forces of acetovanillone on this resin are very weak. The column was washed with 200 ml. of distilled water to remove the acetovanillone. The microcolumn was cut, the top 0.5 inch was extruded and leached with buffer. The spectrum of the yellow material was found to be almost identical with that determined by the first method and both closely approximated that of vanillil. Vanillil was chromatographed on paper and was found to have an R_f value identical with the unknown interfering material.

Table I. Determination of Acetovanillone

Sample Wt., Mg.	Absorbance					% Acetovanillone Calcd. from Pairs of λ		
	4000	3600	3375	3200	2750	4000/ 3375	3600/ 3200	3600/ 2750
56.2	0.128	0.330	0.375	0.338	0.297	0.099	0.102	0.098
70.1	0.159	0.410	0.469	0.429	0.379	0.099	0.101	0.097
59.9	0.160	0.379	0.421	0.379	0.322	0.098	0.100	0.098

DISCUSSION

If oxidized alkaline waste sulfite liquor is chromatographed in the described manner but omitting the bisulfite block, vanillin, acetovanillone, and *p*-hydroxybenzaldehyde move to about the same position on the paper strip. Vanillil overlaps but is slightly higher. The amounts of vanillil and *p*-hydroxybenzaldehyde are so low relative to vanillin that, in the analysis for vanillin, correction need be made for acetovanillone only. (*p*-Hydroxybenzaldehyde is known to be present from electrophoretic analysis.) In the analysis for acetovanillone, with a bisulfite block, the *p*-hydroxybenzaldehyde is stopped and correction need be made for vanillil only.

If the development time is extended from 2.5 to 4 hours, vanillil can be completely separated from acetovanillone. However, within the concentration range studied, the blank from the paper can be a significant part of the total absorbance and variations in the paper blank can cause scattering in the results from replicates. The vanillil present raises the total absorbance and thus reduces percentage variability due to the paper. It has been found more satisfactory to include some vanillil with the acetovanillone in the paper cuts and to calculate out its effect rather than to chromatograph for a longer period of time.

To determine if substances other than vanillil contributed to the absorbance of the solution formed by leaching the acetovanillone from paper, the concentration of acetovanillone was calculated using several pairs of wave lengths as shown in Table I which records the analysis of a sample in triplicate.

It is highly improbable that such a constant value could be obtained over such a wide range of wave lengths if other substances were present that absorbed in the region indicated.

As a check on the recovery of acetovanillone, 10.25 grams of a solution of autoclave product were added to 11.41 grams of a solution consisting of 0.082% acetovanillone in dilute sodium hydroxide. The autoclave product analyzed 0.079%; the acetovanillone analyzed 0.0748%, a recovery of 91.2%; and the mixture analyzed 0.0754%. The mixture should have analyzed 0.0768%—the difference, somewhat less than 0.2%. These data, together with those in Table I, indicate an accuracy within ±5%.

Vanillil may be determined at the same time, if desired by including all of it with the acetovanillone.

ACKNOWLEDGMENT

The authors are indebted to Robert E. Keller for invaluable help and suggestions.

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Received for review August 25, 1954. Accepted November 15, 1954.

Preparation and Half Life of Carrier-Free Yttrium-90

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A rapid method was needed for obtaining a continual supply of carrier-free yttrium-90. The lack of agreement among the reported half-life values indicated that a precise determination of the half life of yttrium-90 would be desirable. Carrier-free yttrium-90 was "milked" periodically from a purified strontium-90 sample by precipitation of the strontium-90 with strontium nitrate carrier in 80% nitric acid. The half life, determined by beta counting, was found to be 64.029 ± 0.024 hours. By use of the recommended procedure high purity yttrium-90 with yields of 95% is available when needed for an indefinite period of time.

RADIOCHEMICAL methods are particularly useful in the determination of yttrium in fractionation and coprecipitation studies (*29*) because many of the physical methods used in rare earth analyses—e.g., absorption spectroscopy, magnetic susceptibility, etc.—are not applicable. Yttrium-90 is a suitable nuclide for these tracer experiments because it has a convenient half life of about 64 hours, is a pure beta emitter which decays to a stable isotope (zirconium-90), and since a continual supply of yttrium-90 can be obtained by "milking" it at desired intervals from its long-lived parent, strontium-90 (19.9-year half life) (*19*).

Although carrier-free yttrium-90 has been separated from strontium-90 by radiocolloidal formation of the yttrium in basic solutions (*2*, *15*, *27*, *30*), by ion exchange (*34*), by electrochromatographic methods (*3*, *25*), by solvent extraction (*4*), by mass spectroscopy (*9*), and by vacuum evaporation (*31*), the authors have found that carrier-free yttrium-90 can be separated rapidly with a high yield and purity by precipitation of the strontium-90 with inert strontium nitrate carrier in 80% nitric acid. The procedure, which is based on an analytical method developed by Willard and Goodspeed (*36*), is similar to that previously reported (*23*) for the separation of carrier-free lanthanum-140 from barium-140. Modifications of the Willard and Goodspeed method have been used by other investigators (*5*, *6*, *16*, *35*) to separate strontium and barium activities from fission product mixtures. Nottorf (*17*) used the fuming nitric acid procedure to separate yttrium-90 with yttrium carrier from strontium-90.

The lack of agreement among the reported half-life values shown in Table I indicated that a precise determination of the half life would be desirable. Accordingly, such a determination was made on a highly purified sample of yttrium-90.

EXPERIMENTAL

In the recommended procedure the strontium-90 parent is first purified. This is accomplished by a nitrate precipitation in 80% nitric acid with inert strontium carrier followed by two homogeneous chromate precipitations (*24*) with barium carrier. The strontium is finally recovered from the chromate filtrate by precipitation of strontium nitrate in 70% nitric acid. The strontium-90 need be purified only once, after which yttrium-90 can be "milked" when needed for an indefinite period of time. The nitrate precipitation in 80% nitric acid removes all possible contaminants with the exception of isotopes of strontium, barium, radium, and lead (*36*). The chromate precipitations remove barium, radium, and lead. Strontium-89, which may still be present as an impurity, does not interfere since it decays to a stable isotope, yttrium-89.

The purified strontium is reconverted to the nitrate and set aside to allow for the regrowth of yttrium-90. The yttrium-90 is then separated from strontium-90 by reprecipitation of the strontium nitrate in 80% nitric acid. Carrier-free yttrium-90 is recovered from the filtrate.

REAGENTS

Ten millicuries of strontium-yttrium-90 equilibrium mixture as the chlorides in dilute hydrochloric acid solution were obtained from the Radioisotopes Division of Oak Ridge National Laboratory. The isotopes were reported to be carrier-free. Strontium-89 (less than 0.1%) was the only radioactive impurity reported to be present. Spectrographic analysis indicated that trace amounts of the following elements were present: boron, chromium, nickel, lead, aluminum, iron, magnesium, calcium, and strontium.

The red fuming nitric acid was Baker and Adamson reagent grade and assayed 93.1% nitric acid and 7.05% nitrogen trioxide. The concentrated (70%) nitric acid, strontium nitrate, potassium dichromate, and potassium cyanate were analytical reagent grade.

RECOMMENDED PROCEDURE

Purification of Strontium-90. Dissolve the desired quantity of strontium-yttrium-90 and 0.75 gram of strontium nitrate carrier in 2 ml. of water. Add dropwise 2 ml. of concentrated nitric acid and evaporate with stirring until the volume of the mixture is approximately 2 ml. Cool in an ice bath and add 3 ml. of red fuming nitric acid. Digest at 0° C. for 15 minutes with stirring. Filter the strontium nitrate and wash three times with 1-ml. portions of 80% nitric acid. Discard the filtrate and washings.

Table I. Survey of Reported Half-Life Values for Yttrium-90

Date	Source	References	Half Life, Hr.
1936	$Y(n, \gamma)$	(*10*)	70
1937	$Y(n,\gamma)$	(*18*)	57.6 (2.4 days)
1937	$Y(d,p)$	(*33*)	60.5 ± 2.0
1938	$Y(n,\gamma)$	(*20*)	66
1938	$Nb(n,\alpha)$	(*21*)	66 ± 3
1940	$Zr(n,p)$	(*22*)	66 ± 2
1941	$Y(n,\gamma)$	(*32*)	60
1946	$Y(n,\gamma)$	(*1*)	61 ± 1
1946	Fission products	(*8*)	72
1948	Fission products	(*9*)	60
1949	Not reported	(*28*)	108 (days)[a]
1949	Fission products	(*7*)	62
1951	$Sr(2n,\beta)$	(*12*)	>60
1951	Fission products	(*17*)	65
1953	$Zr(n,p)$	(*26*)	65
1954	Fission products	(*4*)	64.60 ± 0.43

[a] Probably incorrect mass assignment.

Dissolve the strontium nitrate in 2 ml. of water, and add 20 mg. of barium nitrate and 50 mg. of potassium dichromate. Adjust the acidity of the solution by the dropwise addition of dilute nitric acid and ammonium hydroxide until a slight precipitate of barium chromate just redissolves in 1 drop of dilute nitric acid. Add 0.5 ml. of glacial acetic acid. Dilute the solution to 5 ml. with water. Add 50 mg. of potassium cyanate. Heat to about 60° C. and stir for 30 minutes. Cool and filter, but do not wash the barium chromate. Discard the precipitate. Add 20 mg. of barium nitrate to the filtrate, readjust the acidity with dilute nitric acid and ammonium hydroxide, and carry out a second barium chromate precipitation by the described procedure. No additional potassium dichromate need be added to the filtrate for the second precipitation, as a considerable excess was used in the first precipitation.

Add 2 ml. of concentrated nitric acid to the filtrate from the second barium chromate precipitation and precipitate the strontium nitrate by evaporating the solution to 2 ml. Cool the mixture in an ice bath, filter, and wash the strontium nitrate with a few milliliters of cold concentrated (70%) nitric acid until the washings are free of the orange dichromate color. Discard the filtrate and washings. Set aside the strontium nitrate containing the purified strontium-90 for about 2 weeks to permit the growth of yttrium-90. In the preceding step, strontium nitrate was precipitated from 70 rather than 80% nitric acid because red fuming nitric acid was observed to reduce chromate ion to an insoluble brown oxide.

Separation of Yttrium-90. After fresh yttrium-90 has grown in, dissolve the strontium nitrate in 2 ml. of water. Reprecipitate the strontium nitrate from 80% nitric acid as in the strontium-90 purification. Filter the strontium nitrate, but do not wash it. Evaporate the filtrate to dryness in a small beaker (20 or 30 ml.). Dissolve the slight residue in dilute nitric acid and dilute the solution to the desired volume. Save the strontium nitrate for future milkings.

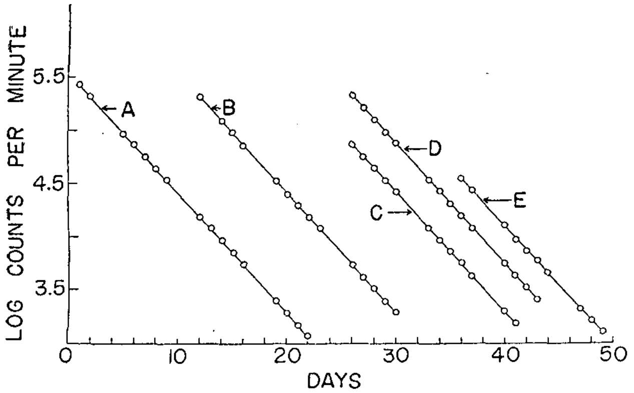

Figure 1. Decay of yttrium-90

Sample	$T^{1}/_{2}$, Hr.	P.E.
A	64.1060	0.0494
B	63.9699	0.0425
C	64.0483	0.0294
D	63.9211	0.0443
E	64.1418	0.0578
Mean	64.029	0.018 (int.) 0.024 (ext.)

The separation can be carried out in small beakers using small sintered-glass funnels for the filtrations or, more conveniently, in a modified filter-beaker described in an earlier paper (*23*). The filter-beakers are particularly suitable since the strontium-90 is confined and need never be removed. In either case, it is suggested that reagents be added with medicine droppers, stirring effected with a magnetic stirrer, and heat supplied by an infrared lamp.

One millicurie is a convenient quantity of strontium–yttrium-90 to use, although larger quantities may be used with suitable shielding. At least 2 weeks should be allowed between successive milkings.

HALF LIFE

Table I shows the need for a precise determination of the half life of yttrium-90. The reported half-life values are listed by date. Yttrium-90 was prepared by a variety of methods: neutron bombardment of yttrium, niobium, zirconium, and strontium; deuteron bombardment of yttrium; and separation from fission products. The half-life values vary from 57.6 to 72 hours with most of the values falling between 60 and 66 hours. The 108-day half life is probably the result of an incorrect mass assignment.

The yttrium-90 used for the half-life determination was first separated and purified by the recommended procedure. A sample was taken for the determination of the yttrium-90 yield and of the quantitativeness of precipitation of strontium nitrate in 80% nitric acid. To the remainder was added inert strontium, and the strontium nitrate precipitation in 80% nitric acid was repeated. The filtrate was evaporated to dryness, and the residue was dissolved in dilute nitric acid.

The yttrium-90 preparation, as calculated from the data on the yield and quantitativeness of precipitation, contained approximately 90% of the original yttrium-90, 10^{-7}% of the original strontium-90, and 20 γ of inert strontium. The residual strontium-90 was also determined by counting after the yttrium-90 had decayed to radioactive equilibrium.

A sample of the yttrium-90 solution was mounted for beta-ray-spectrometer analysis. The beta spectrum was found to be substantially in accord with published values (*11*). Strontium-90 was not detected.

The telescoping technique, by means of which a radioactive material can be counted for many half lives without loss of counting precision, is illustrated in Figure 1. Five samples of yttrium-90 of ascending magnitude were prepared for beta counting. Sample A, which originally contained 0.33 microcurie of yttrium-90, was counted daily beginning 1 day after purification. By the 23rd day, it had decayed to less than 1000 counts per minute, the arbitrary lower limit.

Meanwhile, Sample B, which originally contained 5 microcuries of yttrium-90, had decayed to less than 350,000 counts per minute, the upper limit of the counter. It was counted daily from the 12th to the 30th day after purification. Samples C, D, and E, containing 62, 179, and 350 microcuries of yttrium-90, respectively, were treated in the same manner.

Thus, while no one sample was counted for more than 8 half lives, their composite decay spanned a period of 18.4 half lives. Instrumentation, counting techniques, and mathematical treatment were identical with those previously described (*14*).

Corrections were made for residual strontium-90 in all samples in which the contamination contributed more than 1 count per minute. The half life of each sample was determined, and the five half-life values were examined for consistency. No systematic deviation was observed.

The mean half life, obtained by weighing the five determinations inversely as the squares of their probable errors, was 64.029 ± 0.024 hours (external probable error).

DISCUSSION

The purity of the carrier-free yttrium-90 prepared by the recommended procedure was determined by following its decay. The yttrium-90 showed no significant deviation from a half life of 64.029 hours for at least 26 days, as shown in Figure 2. After this length of time less than 0.2% of the original yttrium-90 remained.

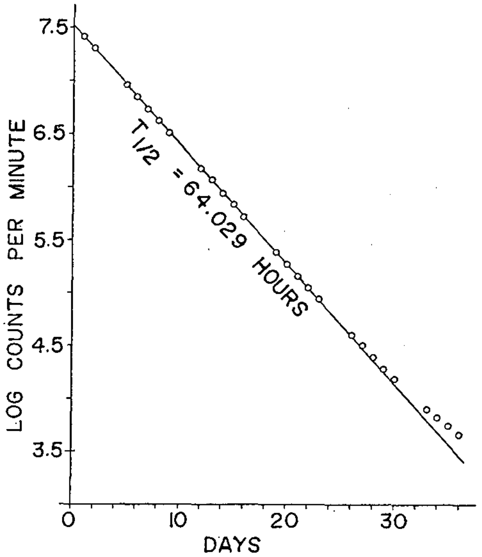

Figure 2. Decay of yttrium-90

Prepared by recommended procedure

A yield of 96% was obtained when the separation was carried out in a filter-beaker. If small beakers and sintered-glass funnels are used, the yield is lower (*23*) due to a greater retention of filtrate on the strontium nitrate crystals. Although washing the crystals in 80% nitric acid would increase the yield, it would also increase slightly the amount of strontium-90 impurity. Therefore, the strontium nitrate crystals should not be washed.

The quantitativeness of precipitation of strontium nitrate in 80% nitric acid was determined by analyzing the filtrates for strontium-90 by the method of differential decay (*13*), and by allowing the yttrium-90 to decay to equilibrium and counting the residual strontium-yttrium-90 activity. The average strontium concentration in the 80% nitric acid filtrates was 0.004 mg. of strontium per ml. Since the total volume was 5 ml., and about 300 mg. of strontium carrier was used, the amount of strontium-90 remaining in the filtrate as a radioactive impurity in the yttrium-90 was about 0.007%. The quantitativeness value for strontium nitrate precipitation in 80% nitric acid solution is approximately the same as that previously found (*23*) for barium nitrate (0.05 millimole of strontium nitrate per ml. as compared to 0.07 millimole of barium nitrate per ml.).

ACKNOWLEDGMENT

The authors wish to express their indebtedness to L. N. Russell and G. R. Grove for their analysis of the beta spectrum of the material used in the half-life determination.

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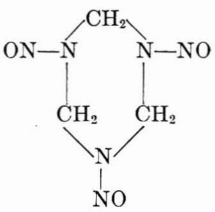
RECEIVED for review May 12, 1954. Accepted November 22, 1954. Presented before the Division of Analytical Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y. Abstracted from Mound Laboratory Reports MLM-937, February 1, 1954, by H. W. Kirby and M. L. Salutsky, and MLM-938, February 1, 1954, by M. L. Salutsky and H. W. Kirby. Mound Laboratory is operated by Monsanto Chemical Co. for the U. S. Atomic Energy Commission under Contract Number AT-33-1-Gen-53.

CRYSTALLOGRAPHIC DATA

93. Hexahydro-1,3,5-trinitroso-*s*-triazine (1,3,5-Trinitroso-1,3,5-triazacyclohexane)

Contributed by RALPH J. HINCH, JR., Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.



Structural Formula of Hexahydro-1,3,5-trinitroso-*s*-triazine

Excellent crystals of hexahydro-1,3,5-trinitroso-*s*-triazine I can be obtained by recrystallization on a macro scale from isopropyl alcohol, amyl alcohol, or benzyl alcohol. The best crystals for optical and x-ray analysis are obtained from benzyl alcohol (Figure 1). Figure 2 represents an orthographic projection of a typical hexahydro-1,3,5-trinitroso-*s*-triazine I tablet obtained by slow recrystallization from benzyl alcohol at room temperature.

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habit. Needles and tablets elongated parallel to *b* lying on the orthopinacoid {100}, upper positive orthodome {$\bar{2}$01}, or lower positive orthodome {20$\bar{1}$} and showing the basal pinacoid {001} and clinodome {011}.

Axial Ratio. $a:b:c = 2.050:1:1.450$.

Interfacial Angles (Polar). 100 ∧ 20$\bar{1}$ = 36°; 001 ∧ $\bar{2}$01 = 56° 35′; 011 ∧ 01$\bar{1}$ = 69° 14′.

Beta Angle. 92° 35′ (reflection goniometry).

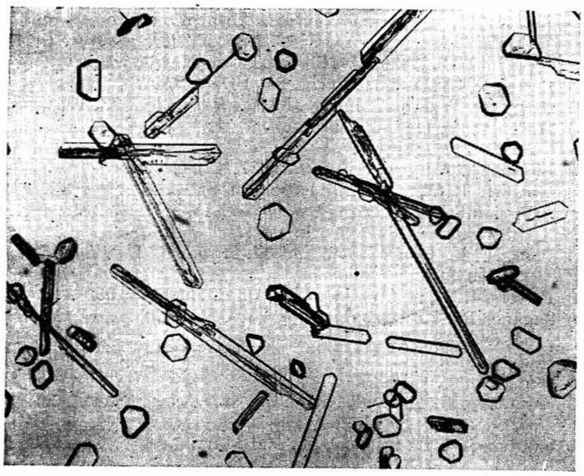


Figure 1. Crystals of hexahydro-1,3,5-trinitroso-s-triazine I from benzyl alcohol on microscope slide

X-RAY DIFFRACTION DATA

Cell Dimensions. $a = 12.83$ A.; $b = 6.26$ A.; $c = 9.08$ A.

Formula Weights per Cell. 4 (3.999 calculated from x-ray data).

Formula Weight. 174.13.

Density. 1.585 (flotation and pycnometer); 1.586 (x-ray).

Principal Lines

d	I/I_1	Index	d	I/I_1
9.06	4	9.07 (001)	2.48	1
6.43	5	6.41 (200)	2.33	3
5.62	1	5.62 (110)	2.27	3
5.37	6	5.35 (201)	2.18	2
5.16	10	5.15 (011) and/or 5.13 ($\bar{2}01$)	2.10	2
4.83	1	4.82 (111)	2.07	2
4.51	7	4.54 (002) and/or 4.48 (210)	2.01	2
3.99	6	4.00 ($\bar{2}11$)	1.957	2
3.77	6	3.78 (202) and/or 3.80 ($\bar{3}01$)	1.926	2
3.67	4	3.67 (012)[a]	1.881	1
3.52	9	3.53 (310)[a]	1.845	1
3.22	6	3.21 (400)[a]	1.778	1
3.13	4	3.14 ($\bar{2}12$)[a]	1.746	1
3.02	3	3.04 (120)[a]	1.725	1
2.88	8		1.667	Very weak
2.76	3		1.613	Very weak
2.70	3		1.560	Very weak
2.65	2		1.449	2
2.58	3		1.361	Very weak
2.54	2			

[a] Other reflections might contribute to these lines and indicated indices are suggested only as possible contributing reflections.

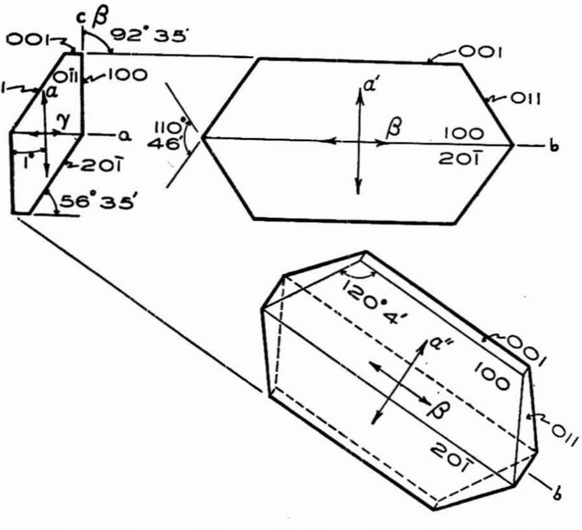


Figure 2. Orthographic projection of typical crystal of hexahydro-1,3,5-trinitroso-s-triazine I

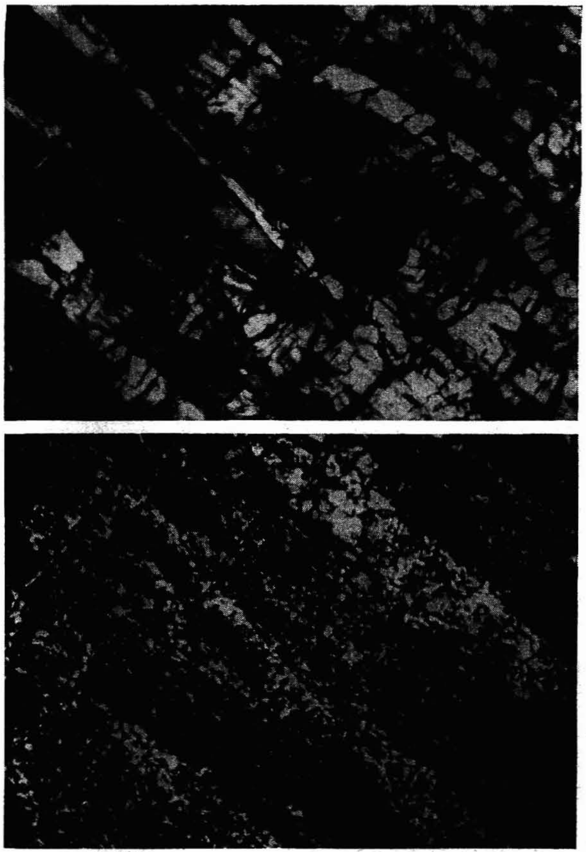


Figure 3. Hexahydro-1,3,5-trinitroso-s-triazine from fusion

Upper. **Modification I recrystallized from supercooled melt (crossed Nicols)**

Lower. **Same field showing modification II after transformation of I to II (crossed Nicols)**

OPTICAL PROPERTIES

Refractive Indices (5893 A.; 25° C.). $\alpha = 1.582 \pm 0.002$. $\beta = 1.607 \pm 0.002$. $\gamma = 1.613 \pm 0.002$.

Optic Axial Angles. $2E = 89°$ (blue); 100° (yellow); 108° (red). $2V$ (calculated from β and $2E$) = 52° (blue); 57° (yellow); 61° (red).

Dispersion. $r > v$, very strong.

Optic Axial Plane. 010.

Sign of Double Refraction. Negative.

Acute Bisectrix. α.

Extinction. $\gamma \wedge a = 3° 35'$ in obtuse β.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.60$. R (calcd.) = 37.9; R (obsd.) = 37.5.

FUSION DATA. Two polymorphic modifications of hexahydro-1,3,5-trinitroso-s-triazine have been observed from fusion. Form I is birefringent; form II is isotropic. On heating, modification I transforms to modification II, the latter melting at 107–107.5° C. The system is enantiotropic. The transformations I → II and II → I occur rapidly a fraction of a degree above or below the transformation temperature, 97° C. Form I shows characteristic shrinkage cracks (Figure 3) when recrystallized from the supercooled melt.

ACKNOWLEDGMENT

This work was performed under an agreement between the Armour Research Foundation of Illinois Institute of Technology and Arthur D. Little, Inc., Cambridge 42, Mass., under Ordnance Contract DAI-19-020-501-ORD (P) 33. Although part of a confidential project, this material was declassified by authority of E.O. 10501 signed by J. R. Browder on January 26, 1955. Acknowledgment is due to Irene Corvin, who determined the powder x-ray spacings and intensities.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

Preparation of Pressed Samples for Counting Carbon-14–Labeled Compounds

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A NUMBER of methods for the preparation of solid samples are in general use for determining radioactivity. Filtration techniques (*1, 3, 5*), centrifugation (*2*), or the removal of a solvent or suspending fluid (*2*) are often satisfactory for plating a substance, but there are instances when it is advantageous to prepare mounts of infinite thickness from a dry solid.

The authors have found it desirable to employ infinitely thick samples in in vivo experiments where respiratory carbon dioxide is trapped, precipitated as barium carbonate, collected, dried, weighed, and counted. Such mounts have been prepared by manually packing the solid material in the planchet with the aid of a small spatula. The method is time-consuming and extremely difficult with most materials, and the problem of obtaining a smooth flat surface with granular solids is also encountered.

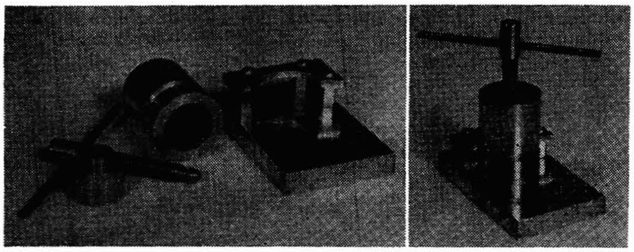

Figure 1. Press for preparing solid mounts

To avoid these difficulties, a small inexpensive press has been designed and built to prepare uniform solid mounts. This method has been tested for the uniformity of the sample prepared, contamination of successive samples, and adaptability to materials other than barium carbonate.

METHODS AND MATERIALS

This press is shown in Figure 1, both disassembled and in its operating position.

The base of the press measures $0.5 \times 2^{3}/_{8} \times 4$ inches. The cylinder has a height of 2 inches and the cylinder bore has a slight recess to accommodate the sample holder. The packing piston is slightly tapered to correspond to the sides of the container. The material to be plated is distributed in an approximately even manner in a 1-inch aluminum planchet and placed in the press, considerable pressure is applied, and the sample is removed from the press. A small amount of material may creep upward along the side of the planchet, but it may be easily removed with the tip of a spatula. Traces of the compound left in plating are wiped from the packing piston between preparations of successive samples. Mounts prepared in this manner display a smooth surface and appear to be of uniform thickness.

All samples reported exceed minimal infinite thickness (*3*), and were counted with both a thin-window Geiger-Müller tube and a windowless gas flow counter. A minimum of 10,000 counts was made on each radioactive sample. When the gas flow counter was used, the mounts were covered with aluminum foil in an attempt to improve counting (*4*). The usual precautions must be observed for handling radioactive compounds. Periodically the press must be thoroughly cleaned.

RESULTS

The radioactivity of samples of carbon-14–labeled barium carbonate measured with both counters is shown in Table I. Two planchets contained samples of nonactive barium carbonate which had been plated directly after active samples and were used to measure any contamination present in consecutive mounts. Similar information as to precision was obtained on four planchets prepared from carbon-14–labeled alanine. The similarity of sample activity noted with both barium carbonate and alanine indicates the uniformity of samples prepared by this method. The procedure is further recommended by its simplicity. Its use becomes practical and desirable when a compound whose activity is to be determined must, for other reasons, be dried and weighed prior to counting. The procedure requires a fairly large amount of active material, which may limit its use.

Table I. Radioactivity of Barium Carbonate and Alanine Samples

No. of Planchets	Sample	Thickness, Mg./Sq. Cm.	Radioactivity			
			Geiger-Müller Counter		Gas-Flow Counter	
			Mean c.p.m.[a]	Standard deviation	Mean c.p.m.[a]	Standard deviation
6	$BaCO_3$	80–130	146	2.0	283	5.6
2	$BaCO_3$[b]	48–66	[c]	...	[c]	...
4	Alanine	32–47	422	0.8	804	8.8

[a] Corrected for background.
[b] Barium carbonate not labeled.
[c] No significant difference from background.

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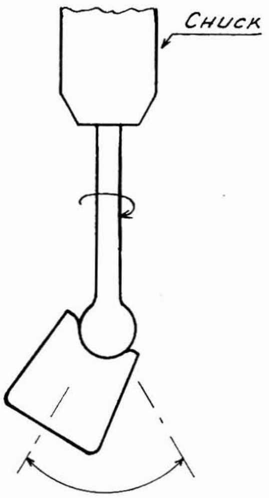
Rapid Preparation of Mineral Oil Mulls

A. K. Presnell, The Andrew Jergens Co., Cincinnati, Ohio

A SIMPLE apparatus for rapid and efficient grinding of mineral oil mulls for infrared spectroscopy may easily be prepared.



A pestle is formed from a borosilicate glass rod about $^{1}/_{4}$ inch in diameter and 4 inches long. One end of this is heated until a roughly spherical portion approximately $^{1}/_{2}$ inch in diameter is formed on it. The enlarged portion should be accurately aligned with the rod.

A mortar is made from a No. 27 standard-taper borosilicate glass stopper or some similar piece of glass. The stopper is strongly heated and a carbon rod is pressed into the flat end to form a depression about $^{1}/_{4}$ inch deep, roughly fitting the pestle. The pestle is mounted in the chuck of a stirring motor and ground to give a spherical fit with the mortar, using No. 180 Carborundum and water.

Grinding with a finer grade of Carborundum might result in still better dispersions.

In use the pestle is rapidly rotated in the vertical chuck of a variable-speed stirring motor, and the mortar is moved over its ground surface from below. Unless very small quantities are used, the material being ground is rapidly forced out, and at intervals of a few seconds must be scraped back onto the grinding surfaces. This can be done quickly, without stopping the motor, by collecting the expressed suspension with a spatula.

The spherical surfaces of the mortar and pestle provide a perfect fit over the entire grinding area. The spherical contact also permits continuous movement of the mortar over the surface of the rotating pestle and thereby avoids an axial dead spot. Grinding is extremely rapid. Good mineral oil mulls of some materials have been prepared by this means in less than 15 seconds of grinding. The degree of subdivision of the particles is greater than the writer has obtained with any other apparatus. The mull is more readily removed from the small mortar and pestle than from larger devices.

Borosilicate glass has been found very satisfactory for the apparatus and there has been no indication of contamination of the mulls by the glass. However, if it were desired to grind hard materials, the apparatus could be constructed of hardened steel. It should be useful for grinding small quantities of materials other than mineral oil mulls.

Photometric Titration Assembly for Beckman Model DU Spectrophotometer

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Institute for Atomic Research and Department of Chemistry,
Iowa State College, Ames, Iowa

The technique of photometric titrations affords a simple solution to many complex analytical problems. Various apparatus for photometric titrations have been described by previous workers. Bricker and Sweetser (*1*) replaced the cell compartment cover with a felt pad through which a buret tip was inserted and carbon dioxide was bubbled through the titration cell to ensure mixing of the reagents. Reilley and Schweitzer (*3*) employed a similar apparatus but replaced the carbon dioxide bubbler with a conventional stirrer. A magnetic stirrer employed in a Beckman Model B spectrophotometer has been described by Hume (*2*). Because of the greater versatility of the Beckman Model DU spectrophotometer, an interchangeable cell compartment was constructed to adapt this instrument for photometric titrations. Alterations of the instrument proper were not required for this modification.

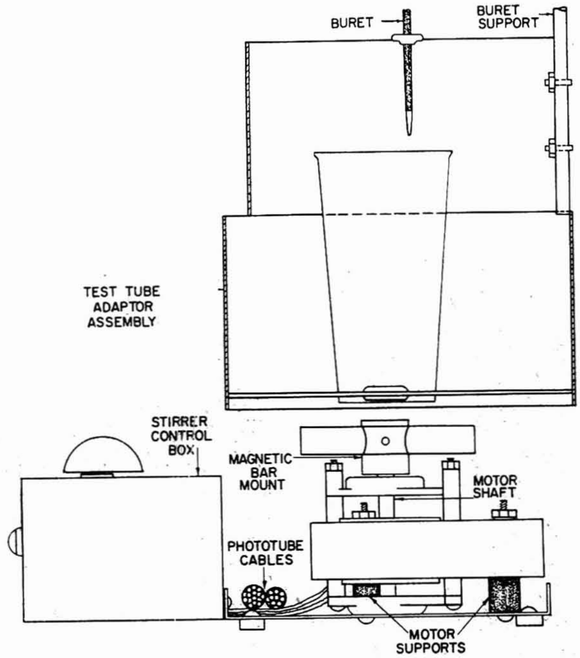

Figure 1. Schematic diagram of titration assembly

A Beckman test tube adaptor assembly for the Model DU spectrophotometer was modified for construction of the titration assembly. A $^1/_4$-inch hole was drilled in the center of the top of the compartment cover and fitted with a rubber grommet of suitable size to permit the light-tight insertion of a buret tip. The test tube holder platform was removed and the two guide rods were used for positioning the titration cell. A $^3/_8$-inch support rod 20 inches long was mounted on the back of the cell compartment cover and equipped with a clamp to support the buret. Thus the buret is mounted firmly on the hinged cell compartment cover. The entire buret below the graduation marks was sprayed with optical black paint to prevent entrance of external light.

A Labline "magne-stir" magnetic stirrer was adapted for use with this attachment. The case was removed and the motor lowered to the base plate by shortening the motor support bolts and spacers. The motor shaft and magnetic bar mount were shortened to give the magnetic stirrer an over-all height of $2^3/_4$ inches, which allowed it to be inserted under the cell compartment. The armature bearing support bolts were changed from steel to brass to eliminate magnetic attraction. The stirrer controls and pilot light were mounted in a 2 × 2 × 4 inch utility cabinet which was bolted to the side of the stirrer base plate. This arrangement made the controls readily accessible at all times.

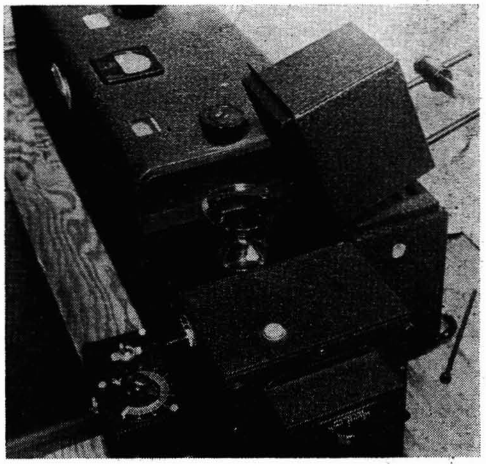

Figure 2. Photograph of mounted assembly

The titration cell for use in the visible region of the spectrum was a 180-ml. electrolysis beaker. The bottom $^1/_2$ inch of one side of the beaker was flattened slightly to permit it to fit firmly between the guide bars. A similar cell for use in the ultraviolet region of the spectrum was constructed from a fused silica tube.

The titration cell, containing the sample and magnetic stirring bar, was placed in the cell compartment and the stirring motor started. The stirring rate was adjusted such that the vortex of the solution did not interfere with the light beam. The addition of each increment of titrant was terminated by rapidly rotating the stopcock from off to on to off. This technique left a small and reproducible droplet on the buret tip.

This photometric titration attachment has been employed in this laboratory in studying chelate kinetics, molar absorptivities, and routine analytical determinations. These particular applications will be described in forthcoming publications.

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— MEMO —

To Research Chemists!

SUBJECT: Organic Reactions Utilizing Organolithium Compounds

NOTE: Recognizing the ever spreading interest in organic reactions utilizing Lithium metal or compounds derived from it, we are for the sixth year compiling and editing the Annotated Bibliography On The Use Of Organolithium Compounds In Organic Synthesis.

The original bibliography was published in January, 1949. A review of literature published prior to January, 1948, it contains abstracts of 223 articles from chemical journals, giving periodical source, date and author.

Supplement No. 1, published in January, 1950, contains 136 abstracts of articles published since the original bibliography.

Supplement No. 2, published in January, 1952 contains another 218 abstracts of literature published since 1950. An index of the principal compounds refers them to the appropriate abstract.

Examples of lithium organic reactions include their use as a condensing agent; as Claisen condensations; alkylation of nitriles and ketones; synthesis of ethinyl compounds and acetylenic carbinols; synthesis of the Grignard type; and preparation of organometallic compounds of other metals.

A request attached to your letterhead will bring you this tool for lithium research. Specify Original Bibliography, Supplements I or II.

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For further information, circle number 13 A on Readers' Service Card, page 43 A

Automatic Operations

GORDON D. PATTERSON, Jr.

E. I. du Pont de Nemours & Co., Inc., Buffalo 7, N. Y.

INCREASING application of known techniques has been the keynote of developments in the field of automatic analysis during the 3 years since the previous reviews on this subject (*340*). Where formerly emphasis was on the prototype design and manipulative details of instruments and measuring elements, we now have the more satisfying situation of extensive commercialization, even competition, as well as much published research based on significant experience. This is not to say that instruments embodying new techniques are no longer needed or being developed, for nearly every major scientific meeting related to chemistry features papers on devices calculated to provide the researcher and the manufacturer with chemical analytical data more reliably and quickly.

The bulk of published accounts during the 3 years up to July 1, 1954, however, have revealed a recognition of the wealth of sensing and measuring elements which the physicist and chemist have already been using in the laboratory. Some of these, necessarily only semiautomatic for research purposes, have been made nearly fully automatic in measuring raw material and product quality. While each is no more (or less) an instrument after this automatization metamorphosis, the attention given the field of analytical chemistry by instrument manufacturers has given rise to the notion that analysts are suddenly beginning to use instruments. Actually little progress is evident in the literature in the development of previously totally unknown measuring techniques. Most cases have been essentially an adaptation or refinement of tools long available for manual operation.

This review continues the outline of its predecessors (*340*) in following the sequence of operations in a normal analytical procedure—namely, sampling, treatment, separation, and measurement, in addition to some comments on general trends and handling of data. As before, greatest attention is given the measurement operations, with brief mention of representative applications of the various physical properties capable of automatic measurement. Unlike previous practice, the reviewer has not attempted to list more than a few commercial instruments in certain categories. While advertising literature is often a worth-while source of information in new measuring devices, the data so offered are usually only partially satisfactory in giving the modern analytical chemist and quality control man the complete picture he needs. The bulk of the references cited have been obtained through *Chemical Abstracts*. In some cases papers are mentioned which have been presented at national American Chemical Society meetings but not yet published.

GENERAL TRENDS

It has been said that future industries will require robots to control robots. The increasing complexity of measuring and control devices leads to greater servicing difficulties and requires automatic features to correct errors of drift and occasional failure. There is an increasing demand that suppliers refrain from using cheap component parts even occasionally and that circuit and mechanical designs be tested out thoroughly before approval for use. More utilization of midget components is giving the word "portable" greater meaning when applied to certain instruments.

There is little doubt that the gap between the available resources of instrumentation and the uses which have been made of them is being narrowed, but there is still a long way to go. The analyst can relegate his problems to the instrument and electronic specialists to a certain degree, but he alone can decide what is required. Hence, the need for the nonspecialist, who understands broadly the techniques available, becomes more critical as the demands of quality measurement and control increase. Enthusiasm for the "black box" can tend to obscure the fact that good judgment is required by one who understands.

Literature. A number of general comments in the literature on progress in the field of instrumentation are worth mentioning. Churchill states his position in "the consumer talks back to the instrument manufacturer" (*75*). Dobson (*106*) has called for greater cooperation between the user and the maker, pointing out that oversecrecy leads to misapplications.

Dever asks the question "How automatic can we get?" (*97*). Tobi has disclosed his views on "the art and science of industrial instrumentation" (*432*). There have been a number of semantic difficulties with both new and old words and definitions. Even instrumentation does not have a single commonly accepted meaning, and "automation" has been used in various ways (*71, 99*). Gallagher (*147*) in a general discussion of modern instrument trends lists analytical instruments and controls in number one place.

A number of books on instruments and their design have been published which should interest those concerned with automatic analysis. Several bear on electronics and electrical measurements (*9, 55, 176*), while others deal with laboratory instruments (*122, 465*) and the new automation (*99*). A new journal, *Instrument Engineer*, appeared in 1952. Aronson's series of papers on instrument electronics (*10*) has included much of direct interest to automatic measurement.

Design trends have been discussed for laboratory apparatus by Müller (*322*), Leroy (*269*), Gutman (*163*), Harris (*175*), and Beckman (*26*); and for electrical instruments in general by Klepp (*247*), Banner (*17*), Hart (*179*), Peterson (*350*), Weaver (*455*), and Dobrin (*104*).

Fyffe and Arobone (*145*) reviewed the status of strain gages and their applications in measurement and control. Various points on servicing and testing are particularly brought out. Questions on "gadgeteering" are raised by Simon (*401*), while Silsbee (*399*) discusses fundamental units. Little (*280*) differentiates between research and industrial instrumentation. Human aspects of instrument design are pointed out by Mangelsdorf (*296*) and by Beals (*24*).

While no inclusive reviews covering all types of automatic analyses have appeared, several more or less general papers bearing on certain fields of analysis may be mentioned. Evidence of increasing use of automatic sampling and measuring techniques is evident in the series of reports on atmospheric pollution studies. A book on air pollution edited by McCabe (*288*) contains chapters by various authors describing equipment and applications. Other reports on air pollution describe the instruments used (*162*, *168*, *236*, *287*), and Kay (*236*) emphasizes the fact that "a new understanding of the important effects of meteorological variables combines with increased laboratory costs to establish the importance of direct-reading continuous techniques, both for control and for environmental exploration work." The related field of river pollution is covered by Hoak (*195*).

Reviews on analytical instrumentation in the petroleum industry include one on optics in the oil industry by Foote (*134*), and others on petroleum quality analyzers by Harrop (*178*) and Pellettere (*343*). Levin's (*270*) review of the analysis of petroleum traces the progress to date and forecasts the likely avenues of future developments. Several techniques for moisture measurement have appeared, including a comparison (*166*) of various instrumental methods.

Approaches to the automatic plant through automatic analyzers have been widely discussed and the extent of experimental progress in the chemical process industries has been reported (*13*, *68*, *105*, *262*). These and other similar reports repeatedly emphasize the need for coordinated use of several measuring techniques in almost any process, rather than reliance on single types of measurement.

It goes almost without saying that the monthly columns on instrumentation in the AMERICAN CHEMICAL SOCIETY journals are "must" reading for anyone in this field. Nearly all are pertinent to points covered in this review, although they are not specifically cited in the bibliography.

Meetings. Hardly a major scientific meeting in any of the sciences fails to include paper(s) on some physical technique of measurement related to quantitative chemical analysis. Many papers presented at these meetings are not published for many months afterward and sometimes not at all. Various local ACS sections also have been scheduling lecture series covering aspects of automatic analysis which may or may not be published.

THE SAMPLE

Increased concern over the question of whether or not the material being measured is a truly representative portion of the main product is evident from the numerous publications on sample-taking and measuring devices. It is often very difficult to arrange dependable continuous sampling systems, and much effort is being put into designing samplers with minimum time lags and controlling or compensating sample temperature, etc. In the case of liquid samples, viscosity fluctuations may be a vital point to consider.

Much significant work on automatic sampling has been reported in papers primarily concerned with the measuring step. Most of these references are cited under the appropriate measurement section, but a few deserve special mention because of novel or unusually complete consideration of the sampling problem.

The term "sampling" is often confused with separation. For example, removal of solid matter from stack gases is a separation process, not sampling, as the specimen so obtained (a solid) is not identical with the original system (the smoke). Likewise, fraction collectors (for chromatographic columns and the like) are not considered sampling devices in this review but a part of the separation process.

As would be expected, the current activity in air-pollution research has been accompanied by a variety of air-sampling devices. In fact, Dieringer and Ingram (*100*) in a review of methods and instrumentation for air sampling stated that the primary problem in air-pollution analysis is the sampling operation. Adams (*1*) described air samplers which operate continuously requiring servicing by an operator only once during each 8-hour shift. Silverman (*400*) described the important methods of sampling stacks for pollution control and summarized in tabular form the significant features of many stack-sampling instruments. Holt (*203*) described a device for studying dusts in the atmosphere.

Thomas and Martin (*426*) and Spracklen (*411*) discussed continuous analytical sampling for vapor phase analysis in the petroleum industry. Samplers developed for more specific purposes have been described in connection with semiautomatic methods for fluoride in air by Thomas (*429*) and for carbon disulfide in air by Treiber and Koren (*433*). Another gas sampler for special applications was developed at the National Bureau of Standards (*307*) for research on the mechanism of engine knock. It can sample a rapidly changing gas over extremely short intervals of about 0.2 millisecond. McGlashen and Powell (*291*) built a device for taking samples of high-pressure gas transmission lines at uniform rates for periods as long as a week.

Barr (*19*) describes several types of continuous analytical sampling in the petroleum industry for both liquids and vapors. Gex and Snyder (*152*) in a modification of previously used odor-threshold devices stress the need of a large continuous flow of the odorous sample to minimize the effects of condensation and contamination.

In the field of liquid sampling, pollution studies are again the prime moving forces for better automatic samplers. Sampling devices for water-pollution surveys have been described by Thomas *et al.* (*428*), Black (*36*), Kline (*248*), Wilkinson (*463*), Wilkinson and Briggs (*464*), and Swearingen (*419*). Some new designs feature adaptability to taking one integrated sample over periods ranging from a few minutes to 36 hours, or to taking separate samples at regular intervals over long periods of time. Considerable attention has been paid to the desirability of making truly portable self-contained samplers, featuring battery operation, spring-wound timers, etc. Cooke (*78*) has designed a special continuous-flow cell for use in light absorption measurements on solutions which fade. Lokonov (*283*) has reviewed automatic devices for sampling ores and concentrates.

Treatment of Sample. This year's review includes this section on treatment of the sample to emphasize a growing recognition of the need for automatically conditioning the sample for the subsequent separation or measurement operations. Conditioning may involve only bringing the sample to a predetermined temperature, which may be accomplished through proper design of the sampling device; or it may mean adjustment of pH, which is a control problem in itself. Treatments involving steps leading directly to separation are discussed under Separation.

Many times in routine manual analysis, some operation in treating the sample will be a tedious repetitious step, and occasionally one finds applications of automatic devices designed to relieve the analyst from the strictly repetitive portions of the procedure, even though the remainder may remain manual.

Thus, we have constant liquid feeding and automatic pipetting devices (*5*) such as those described by Maude (*303*), Lehmann (*268*), Birket-Smith (*33*), Anderson (*6*), and Wooster *et al.* (*477*). An automatic test tube washer was described by Parks *et al.* (*338*), and a pipet washer by Barrett *et al.* (*20*).

Reagent addition is an inherent part of several methods. For example, Marks' patent (*299*) covering the automatic determination of available chlorine in water calls for the addition of a potassium iodide solution followed by passage of the mixture through a coil to provide sufficient time for the stoichiometric release of iodine, which is then measured in a depolarization cell. Control of pH is also essential in this method. A similar approach was used by Littman and Benoliel (*281*) in their continuous oxidant recorder. In this case ozone in air is caused to react with potassium iodide solution and the liberated iodine is measured photometrically. The potassium iodide solution is regenerated by passing it over activated carbon.

Haines and Heindel patented (*165*) a method for determining halogenated hydrocarbons in air. To determine methyl bromide the continuously flowing sample is mixed with excess hydrogen, then passed into a 700° furnace. The hydrogen bromide thus formed is then absorbed in water and the electrical conductivity measured. Gex and Snyder's odor-measuring device (*152*) requires a large continuous flow of odor-free diluting air, which is mixed with the odorous sample in varying proportions.

Käuflein (*233*) determined phosphine automatically in acetylene by passing the gaseous sample through an oxidizing solution (iodine in methanol-water) to form phosphoric acid and then measuring the change in pH. Goodman's (*155*) urinalysis apparatus (for sugar and alumin) is another robot, carrying through the complete cycle of the treatment operations, including the addition of reagents, heating, and flushing with fresh water.

Work continues in automatic combustion techniques. Papers on carbon-hydrogen determinations utilizing automatic combustions have been published by Mangeney (*297*), Mitsui (*315*), Sakamoto (*378*), Levy *et al.* (*271*, *272*), and Hozumi *et al.* (*209*). Determination of nitrogen in organic compounds by automatic combustions is reported by Levy and Mathieu (*272*), Mitsui (*315*), Sakamoto (*378*), Gysel (*164*), Unterzaucher (*440*), and Večeřa (*444*). Methods for sulfur and halogens are described by White *et al.* (*461*) and by Sakamoto and Kaneko (*379*).

THE DESIRED CONSTITUENT

SEPARATION

Separations in analytical procedures, while avoided when possible, are frequently essential and can be done automatically in some cases. Previous reviews in this series have emphasized this point, and the literature during the past 3 years provides additional evidence for continued activity in automatically removing either the desired constituent or some interfering constituent(s).

A host of automatic fraction collectors for use with chromatographic columns have been described, as has also the combination of chromatographic separations with continuous measurement of some physical property of the eluate, or a scanning type of measurement without elution. Little need be said of the automatic fraction collectors (*29*, *40*, *50*, *60*, *67*, *102*, *112*, *119*, *128*, *193*, *227*, *274*, *292*, *306*, *353*, *355*, *359*, *362*, *386*, *387*, *443*, *469*) other than that some are mechanically or chronometrically induced, others photoelectric, others gravimetric or siphon-activated, and still others of the drop-counting or electric contact types. In some cases equal-volume or equal-weight fractions are desired, and in other instances fractions in varying amounts dependent on the change in some physical property will be required. Such fractions, of course, may equally well come from an ion exchange or distillation column.

Other automatic features for chromatographic separations generally involve measurement of some physical property of the column or the eluate, such as radioactivity (*38*), visible light absorption (*323*), ultraviolet absorption (*96*), infrared absorption (*208*), electrical conductivity (*109*), polarographic current (*241*), pH (*228*), and refractive index (*434*).

The completely automatic chromatographic separation is still rare, however, and more work is needed of the type that Donaldson *et al.* (*107*) did in arranging to deliver solvent to the column automatically while gradually increasing the solvent's polarity to achieve the separation of organic acids on silica gel. Another step toward more nearly automatic separation was reported by Mazza (*306*), who separated rare earth elements on ion exchange resins in an apparatus consisting of a glass tube (containing the resin) having an attached U-tube which maintains a constant level of liquid in the column. By using a Mariotte bottle he was able to get a rigorously uniform movement of liquid across the column.

Sorption methods other than chromatography may include absorption of a gas into a liquid such as carbon dioxide into water in a gas analyzer patented by Richardson (*360*) or fluoride into water in a modification of the sulfur dioxide autometer described by Thomas (*429*). A German patent (*151*) discloses a method for continuous analysis of commercial oxygen, involving removal of the oxygen by absorption in a liquid. Spracklen (*411*) discusses the use of filters and scrubbers in continuous petrochemical analysis. Sorption of the desired constituent onto a solid followed by desorption is the basis of a gas chromatography technique described by Griffiths *et al.* (*159*), as well as Bertil Hammar (*32*), who worked primarily with hydrocarbon liquids. Chamberlain *et al.* (*66*) described a reciprocating mercury pump for gas analysis.

Extractions of various sorts have been automatized for liquid-liquid systems by Danieley (*89*), Holliman (*200*), Harman (*174*), and Meinke and Anderson (*309*). An automatic drive for a countercurrent distribution apparatus has been designed by Perry and Weber (*347*). Morris (*320*) has described an automatic apparatus for solid-liquid or liquid-liquid phase countercurrent distribution. It provides automatic equilibration, filtration, and transfer of the filtrate to the next unit.

Separations of solids from gases are achieved by passing smoke through constantly moving filter paper strips in instruments described by Hemeon (*191*) and by Delgass *et al.* (*94*). Hirst (*194*) collected spores by using a suction trap in which the spores entered a narrow orifice and were impacted on a Vaseline-coated glass plate. An automatic recording sedimentation balance has been described by Suito and Arakawa (*416*), who found it usable for determining the particle size distribution curves in the range 0.5 to 40 microns. Turnbull and Harrison (*437*) applied the principle of differential permeation through palladium to separate and determine small amounts of hydrogen in nitrogen.

Separations of several types depending on electricity have been automatized. Electrodeposition by automatically controlled cathode potential has been newly described by several authors (*136*, *219*, *279*, *314*, *331*, *335*). Electrostatic precipitators for particulate matter in air (*206*, *282*, *293*) and for aerosols in air (*468*) have been used widely. A fast, continuous dialyzer (*374*) for hydrophilic colloids can free 200 cc. of hydrophilic sol per hour from electrolyte. Several improvements in continuous electrophoresis separations have been published (*44*, *111*, *366*). These have been applied to separations of proteins in blood serum, various amino acids, and barley albumins and globulins. Continuous electrochromatography is the subject of a paper by Sato *et al.* (*381*).

Evaporation or distillation separations continue to be used, but little new research of significance has been published. Garrison and Rolfson obtained a patent (*149*) for an automatic distillation apparatus for petroleum products which draws the temperature-distillate volume curve. Lefkowitz (*267*) described a completely automatic control system for distillations. Hamilton (*171*) described a continuous mercury still. Two papers have mentioned automatic evaporation of solutions, both (*108*, *274*) in connection with radioactive solutions which were to be dried prior to radioactive measurement. One (*108*) has essentially a balanced arm, on one end of which is an electric hot plate carrying the evaporation tray with about 0.5 ml. of the solution. Mounted above the tray is a feed reservoir, the outlet closed by a ground glass which is raised and lowered electrically as the solution is evaporated. There are six of these units, each evaporating about 10 ml. per hour.

Chemical precipitation still is not done automatically, although Garcia (*148*) described semiautomatic equipment for precipitation with hydrogen sulfide in qualitative analysis, and Singh (*403*) designed an automatic leaching apparatus for intermittently washing precipitates or soil.

GRAVIMETRY

Wider use of automatic analytical weighing has been evident from research reports on a variety of studies. Davies (*90*) has reviewed developments in balance design over the past 25 years. Work with the thermobalance has been continued by Duval and coworkers (*103, 113–115*), Kawagaki (*235*), Kinjyo and Iwata (*245*), and Borrel and Paris (*39*). Similar balances have been applied to the study of mineral samples undergoing decomposition by Mauer (*304*) and to the air oxidation of coal (*74*). A differential thermobalance described by de Keyser (*93*) plots the curve of the weight loss–temperature derivative as a function of time (or temperature). It consists of two tubular electric ovens in which two identical samples are kept at a constant-temperature difference as the absolute temperature is increased.

An automatic balance has been described by Brown *et al.* (*47*), who used commercially available components to obtain weight-time records for rates of absorption of a gas on a solid and for determining the optimum conditions of drying precipitates to constant weight, with a sensitivity of 0.1 mg. and a full scale range adjustable from 25 to 100 mg. or more.

Allen and Wright (*4*) designed a photoelectric device for use with Chainomatic-type balances for continuous measurement of density changes in nylon polymers at high temperatures. They were able to follow weight changes of up to 10 mg. per minute with a precision of 1 mg. The balance chain was driven by a slow-speed reversing relay operated through a photocell and amplifier. A black paper mask attached to the pointer intercepted a light beam and direct recording was said to be feasible. Sarakhov's (*380*) high-sensitivity balance is a quartz-thread torsional-type featuring magnetic compensation of the torsion angle and photoelectric reading of the zero position. This design permits an accuracy of about 8×10^{-8} gram, and a total weight change of 2.5 grams is measurable. It was applied to the measurement of the adsorption isotherm of benzene vapor on highly dispersed quartz.

An automatic weight loader for fine balances was described by Hodsman and Brooke (*196*), who devised a method of applying known variable forces to the swinging beam by using the repulsion between a metal plate on the beam and an adjacent rigidly mounted alternating current coil. Weight loading up to 10 mg. was achieved with an accuracy of about 0.2 mg.

As more specific examples of applications of recording balances the following may be mentioned. Trenner *et al.* (*434*) measured the mass of effluent from a chromatographic column with a strain-gage circuit in such a way that the refractive index could be recorded *vs.* weight. Suito and Arakawa (*416*) studied particle size distributions of powders in an automatically recording sedimentation balance (0.5 to 40 microns). Spinedi (*409*) used a recording balance to study electrochemical effects (anodic dissolution) on metallic samples—e.g., brass—at various voltages and current densities.

Beaujard *et al.* (*25*) devised a system for studying equilibria and reaction rates at high temperatures of solids and gases based on a technique of continuous weighing. Simons *et al.* (*402*) built a magnetic weighing and gas density balance. The circuit was designed with a null-point indicator with feedback from the indicator to the weighing magnet. The balancing current was linear with the force applied to the arm of the balance, so that the current required gave a direct reading of the weight. Sensitivities to weight changes of 10^{-7} gram were obtained.

VOLUMETRY

Progress in making strictly volumetric measurements automatically has been scarce, automatic titrations being discussed under Titrimetry. Several techniques involving volume changes in solids which deform in the presence of certain materials have been described. Thus, Krauch's German patent (*254*) reveals the use of polymeric electrolytes to measure pH. For example, polyacrylic acid is said to have stretched molecules in alkaline solution but is coiled in acid solution; hence the volume changes. An instrument based on the resultant variations in length of a foil or fiber containing polyacrylic acid is described. A controller based on this principle can be used with polyhydric alcohols to open and close contacts automatically to maintain desired pH in aqueous solutions.

Case (*62*) has reviewed new moisture-sensitive elements (wood) for measuring relative humidities. An unusual application is described by Schmidt-Nielson (*384*) in studying desert rats, where a tiny humidity-sensitive hair hygrometer was tied to the rat's tail and dragged into the burrow. The relative humidity was recorded on a small smoked glass disk which could be read later in a microscope.

Gases are measured volumetrically in two devices, one described by Cullen (*85*) where the core gas evolved from foundry cores upon decomposition of the organic binder was measured. Ignition was accomplished at constant pressure in an inert atmosphere, and both the rate of gas evolution and the total volume were automatically recorded. Erikson *et al.* (*126*) described an automatic spirometer for complete volumetric recording of respiratory exchange in man. The record obtained gave oxygen consumption, respiratory quotient, per cent oxygen used from inspired air, and per cent carbon dioxide in the expired air.

DENSIMETRY

Gas densities can be measured by gravitometers discussed by Quirk (*356*). Lefevre (*266*) has designed a "baroscope" for continuous measurement of gas density in which density changes operate a shutter arrangement located between a light source and a photoelectric cell. Changes in the cell current were recorded continuously as a measure of the density. At a flow rate of 250 liters per hour, the sensitivity was about 0.01 kg. per cubic meter in the range 0.250 to 0.550 kg. per cubic meter. An apparatus (*125*) for the continuous determination of carbon dioxide in a carbonation gas directs the gas flow into a glass jar equipped with a sealed float to which a scale is attached. Kistiakowsky (*246*) measured densities in gaseous detonation waves using soft x-rays.

Liquid densities can be measured electromagnetically by a method published by Honick (*204*). The measurement is actually of the minimum electromagnetic force, in terms of the current, required to attract an immersed float through a fixed distance. The float contains a soft iron armature and has a specific gravity just outside one of the extremes to be measured. Remote indication or recording is achieved by a conventional meter or recorder. Holmes (*201*) described a direct-reading specific gravity apparatus based on the relative heights of rise in two tubes containing a standard and the sample, both having the same negative pressure applied at the tops. Persyn (*348*) described a continuous analyzer for measuring the strength of sulfuric acid solutions, based on a hydrometer.

MANOMETRY

Pressure measurement has become so highly developed as an aid to control that little research in significantly new automatic analytical applications has been reported recently. As an example of what can be done, Milligan (*313*) has patented a device for the continuous determination of the water content in oil, or of propane, ethane, etc., in oil. It measures the drop of pressure across a fixed-flow resistance tubing, aided by a liquid metering valve. For the determination of water the constant temperature is set at 110°.

Thomas and Lichtin (*427*) have utilized an inexpensive recording differential manometer for reaction-kinetic measurements. A U-shaped capillary tube containing the mercury has one arm interposed in the optical path of a fluorescent lamp light source. The record is made by means of the image of the mercury which is focused onto photographic paper mounted on a moving drum. Wold (*475*) has described a method for determining the respiratory quotient which measures oxygen manometrically and which worked well on yeasts.

VISCOMETRY

This is another field where relatively little automatization research activity of analytical interest is evident in the literature. Wesp (*460*) and Vieweg and Wesp (*445*) have described new rotating cylinder viscometers. One is the compensation viscometer with constant shear velocity and the other an induction motor viscometer featuring constant moment of rotation. The first design calls for two concentric cylinders, the outer one being rotated at a constant speed over wide ranges and the inner one suspended so that it can rotate freely. Any motion of it causes a change in the output of a photoelectric cell in the path of a spot of light reflected from a mirror on the suspension. This suspension also carries a magnet to compensate for terrestrial magnetism. The solenoid current necessary to maintain the suspension at its equilibrium position was a measure of the viscosity. The other design had a large flat metal disk on the suspension which passed between two coils, and the induced current was measured. This viscometer was used to follow the changes of viscosity of 3 parts of polyvinyl chloride in 100 parts of dioctyl phthalate. It was found that at 75° or 85° C. there was little change with time, but at 100° or 125° the viscosity rose rapidly as dissolution progressed.

Fritzsche (*142*) in a general paper on viscometry classifies usages and describes the proper instruments to be used for various viscosity types of petroleum. Bárány *et al.* (*18*) designed a recording viscometer especially for the study of rapid viscosity changes. It consisted of a pump and mixing chamber for rapid mixing of reagents in fixed proportions. A vibrating plunger dipped into the mixing chamber and the changes in amplitude of the vibrations were measured oscillographically. Roth and Rich (*369*) have discussed the general theory of the ultra-viscoson, which employs a pulsed resonant exponentially damped magnetostrictive strip, and present typical applications.

Yanikoski (*478*) has patented a gas analyzer for continuous determination of a gas in a mixture. It utilizes the behavior of a gas whose viscosity apparently changes when the width of the flow path is reduced to values approaching that of the mean free path.

MAGNETOMETRY

A large increase in the design and use of paramagnetic oxygen indicators and recorders is evident in recent publications. Several authors (*124*, *276*, *361*, *389*) have reviewed oxygen measurement in general, with emphasis on the merits of magnetic means. At least three patents (*133*, *240*, *308*) describe paramagnetic gas analyzers.

Foley and Cherry (*133*) disclosed one, primarily for determining the oxygen content of vehicle gas, which has an elongated temperature-sensitive resistor in a tubular gas cell, having its longitudinal axis offset from the surface defined by the lines of the field of maximum density between the magnetic pole faces located on opposite sides of the cell. The gas enters the tube and recirculates in convection loops of width corresponding to the length of the resistor. This flow has a cooling effect on the resistor which is a function of the concentration of the paramagnetic component (oxygen). The resulting resistance change is measured and recorded.

The apparatus patented by Kells and Larsen (*239*) comprises a chamber within which there is an alternating-current magnet having a gap in which there are mounted movable armatures. A pickup connected to the armatures measures a voltage which is amplified and indicated.

Medlock's patent (*308*) covers an apparatus having two cells, one an analysis cell horizontally mounted and having an alternating current bridge circuit, the other a reference cell similar to the first except that it has no applied magnetic field across it and that it is vertically mounted. This second cell, being vertical, has a gas flow which results from the convectional effects of a center-tapped heating winding around it, and this flow is a function of the viscosity of the gas. The temperature difference between the two halves provides additional information on the specific heat and viscosity of the gas.

Ebbinghaus (*116*) has modified the cell design to an annular chamber which permits a strong suppression of the zero point by dynamic compensation of the magnetic wind which occurs in a horizontally mounted electrically heated glass tube in an inhomogeneous magnetic field. Accuracies of 0.01% oxygen were achieved in the range of 20 to 21%. Further modifications to the Magnos magnetic oxygen recorder were made by Ebbinghaus and coworkers (*117*) to make it applicable to biological materials. They were able to determine yeast respiration with and without the presence of sugar.

Hoover *et al.* (*205*) used magnetic oxygen measurement to study aerobic oxidation rates and stated that it should have many applications to research in water supply and waste-treatment processes. Specifically they determined the consumption of oxygen by milk waste.

Griffith (*158*) used the magnetic determination of oxygen as the basis of control of a catalytic purification plant. The instrument was used satisfactorily in the automatic control of a plant designed to remove organic sulfur compounds catalytically from coal gas. The control of oxygen in combustion gases from glass furnaces was accomplished by Naumann (*330*) by a specially designed magnetic oxygen meter.

Behrmann and Hartman (*27*) found that the removal of carbon dioxide in exhaled air caused an apparent increase in oxygen content as measured by the Beckman meter and were able to use it to determine carbon dioxide rapidly.

Applications to solids have been described by Eisler *et al.* (*121*), who determined the ferromagnetic phases present in an iron catalyst for hydrocarbon synthesis. The apparatus utilized an electromagnetic feedback to balance the force on a sample placed in a nonuniform magnetic field.

THERMOMETRY

Temperature measurements can be very useful in certain applications where melting or boiling points or differential thermal analysis curves can be related quantitatively to the chemical content of the material in question. This section includes only such methods, and techniques involving temperature changes due to phenomena such as heats of reaction are covered in the following discussion of Calorimetry.

Several authors have published results of studies on thermal analysis. Cunningham *et al.* (*86*) designed an automatic thermal analysis recorder which produces a series of straight lines so placed that the position of each line represents a set temperature, and the length represents the time the sample required to change from the previous temperature—that is, the line length is the time required to pass through a scientific thermocouple electromagnetic force interval and is a function only of the specific heat or heat transfer rate when no transformation in the sample is occurring. If there is a transformation taking place, the time intervals change, and the temperatures characterizing such transformations can be determined to within 0.2° C.

Penther *et al.* (*345*) built a semiautomatic thermal analysis apparatus for studying transition changes in materials such as soaps, waxes, and greases. Linear heating rates from 0° to 5° per minute and four cooling rates from 0.5° to 4° per minute can be selected. The temperature difference can be recorded either at a constant rate or as a function of the sample temperature (up to 400°). The versatility which makes it so valuable derives from the linearity of its operation during heating and cooling, the special calorimeters which can be sealed off from the atmosphere, and the high sensitivity.

Müller and Stolten (*324*) described the use of thermistors in the precise determination of temperature differences as small as 0.0001° in the thermometric determination of molecular weights. The thermistor resistance changes unbalanced a Wheatstone bridge having a vacuum-tube voltmeter as a detector. Spurious bridge unbalance due to other causes was reduced by having two thermistors in parallel, so that the bridge really functioned as a differential resistance detector. Molecular weights determined in various solvents were found to agree with theoretical values within 1%.

Barshad (*21*) discussed the problems of calibrating a differential thermal analysis apparatus. Spinedi (*410*) built a precision apparatus with recorders which register both the rate of heating of the furnace and the differential curve. Theron (*424*) built an improved automatic apparatus for the differential thermal analysis of minerals with an electronically controlled heating program having a rate between 0° and 50° per minute up to 1100° at an accuracy of ±1°.

New techniques in measuring melting points use optical methods. Furst and Shapiro (*144*) described a device for measuring changes of light transmittance with temperature. The melting ranges thus obtained, while not thermodynamically defined, were very convenient for product purity measurement. Curves plotted showed per cent transmittance *vs.* temperature, and reproducibility was good for samples melting below 230° C. Shapiro's patent (*395*) disclosed the details of construction. Müller and Zenchelsky (*325*) designed an apparatus for automatic determination of the melting point in which the proper temperature is indicated by the increase in light reflected from a polished platinum surface supporting the sample as it melts. The amplified photoelectric response to this increase in reflection stops a servo-potentiometer, thus indicating the temperature attained.

Shipe *et al.* (*397*) devised a semiautomatic cryoscope for determining the freezing point of milk, an important criterion of adulteration. A cooling bath is maintained at a constant temperature by mechanical refrigeration, and the sample is stirred and the thermometer tapped mechanically, thus eliminating the two most tedious steps.

Dew points, of course, are commonly measured automatically as measures of the water content of gases, but Francis (*138*) has used it to measure the sulfuric acid vapor content of combustion products. He found that the presence of a few parts per million of sulfuric acid vapor raised the dew point considerably above that expected with water vapor alone and that the effect of other constituents was negligible. He described an apparatus measuring the dew point by the increase in the electrical conductivity of a glass surface on deposition of a liquid film of an electrolyte, such as sulfuric acid.

Boiling points are closely followed as indications of the progress of distillations and the identity of the vapors being distilled. Shands (*394*) patented a new boiling point indicator featuring a closed testing circuit in which the pressure and temperature prevailing at the sampling point were automatically maintained. The instrument recorded crude product boiling points and returned the test sample to the system. It was especially designed for systems under pressure or moderate vacuum. Heitler (*189*) makes use of a thermistor in following a reaction at the boiling point of a solution. The thermistor serves as one arm of a Wheatstone bridge, and the out-of-balance current, amplified, gives a continuous record of the boiling point of a solution within 0.001°. The device can be used to determine molecular weights with precisions of 0.7% (for 0.2-gram samples).

Flash points are measured automatically in an instrument described by Jacobs (*223*). It determines whether a sample of oil will flash at a predetermined temperature with an accuracy comparable with the best laboratory methods in about 2.5 minutes after the sample has been loaded. A method of moisture control on paper machines, according to Lees (*265*), depends on a differential measurement analogous to wet- and dry-bulb temperatures. The primary detector is a differential thermocouple which measures the difference in temperature between a drying cylinder surface and the paper surface some distance beyond this point. The measured difference results from evaporation, which is related to the paper's water content, being linear between 0 and 8%.

CALORIMETRY

Reactions in which one of the products is exothermal heat have long been used in quantitative determinations. Of course, flammable gas detectors are not new, but patents continue to be issued and novel improvements have been described. Taylor (*422*) has discussed an instrument of the thermocouple type which has increased stability and reduced zero drift. Cotton (*82*), Page (*334*), and Tiffany (*431*) have patented gas detectors, in all of which a catalyst causes ignition of the sample, and the heat evolved is measured by a thermocouple or by the resistance change in a platinum wire.

A modified but basically similar device, covered in a British patent (*202*), determines low percentages of oxygen in combustible gases. Oxygen is determined somewhat differently by Imai (*212*), who has patented a catalyst for oxygen measurement which is prepared by absorbing hydrogen on silica gel and then immersing it in palladous chloride solution. The filtered and dried product evolves heat in the presence of small amounts of oxygen. Engelhardt (*124*), in a study primarily of the paramagnetic determination of oxygen, found that concentrations too low for accurate measurement in this manner (0 to 0.05%) could be measured very nicely by the temperature rise due to absorption.

Kudo and Ihara (*257*) were able to estimate traces of carbon monoxide continuously and automatically in gaseous mixtures of methane, hydrogen, and nitrogen. The sample passes through a catalyst chamber to remove traces of oxygen and sulfur and then is heated to 300° to 400° in a reaction cylinder, the upper part of which is made of brass and the lower part of low-heat-conductivity steel. In the presence of the proper catalyst, the carbon monoxide reacts exothermally with the hydrogen and the resulting temperature increases are measured with a thermocouple having at least 15 contacting points.

Cossey(*81*) has discussed the Cutler-Hammer recording calorimeter. Johnson and Wehner (*230*) have patented a field method and apparatus for determining the amount of Impregnite in cloth. The cloth sample is stretched tightly over a thermopile and treated with phenylhydrazine. An attached galvanometer indicates the heat liberated which is dependent on the Impregnite present.

TITRIMETRY

The very desirable features of automatic titrations have resulted in continued activity in the research and application of a variety of end-point detection techniques and methods of titrant addition. Much activity has centered on coulometric titrations, and work by Carson, DeFord, and Lingane has advanced both the instrumentation and application aspects of this field.

Carson (*61*) devised a titrator for microtitrations using electrolytically generated titrants with a current power supply generating from 10^{-9} to 2×10^{-7} equivalent per second. At the end point the indicator electrodes shut off the current to the generating electrodes, and Carson was able to use a modified pH meter as an impedance match between the indicator electrodes and the trigger input of the controller. The controller also has a timing circuit which avoids false end points by timing the duration of the end point potential. After an apparent end point persists for the correct length of time, the shutoff is actuated. In the titration of uranium with ferric iron the end-point duration time was set at 30 seconds. Such titrations can much more readily be carried out at elevated temperatures and in inert atmospheres than can normal titrations requiring external addition of titrant solutions. Carson has also devised a system for universal anticipation of end points in general automatic titrations. It employs a solenoid-operated glass syringe to remove 10 to 20% of the sample before starting. Then titrant is added rapidly to a predetermined potential, at which point the withdrawn portion of the sample is returned, and the remainder of the titration proceeds slowly to the true end point. Dichromate titrations, where the end-point lag is large, were found to benefit especially by this procedure.

DeFord's review (*92*) of instrumentation for coulometric titrations included current regulators, various end-point detection and anticipation devices, and termination units. Lingane described automatic titrations with electrolytically generated silver ions (*277*) and a constant current source for coulometric titrations (*278*) which maintains electrolysis currents, of any desired magnitude, constant to ±0.01% for several hours. Landsberg and Escher (*260*) have described the Titrilog anew.

Wise *et al.* (*472*) designed an automatic coulometric titrator with photometric detection of the end point. Three types of color changes at equivalence were investigated, and samples of 0.2 to 1 meq. were titrated with average errors of 0.06 to 0.15%. The main idea was to avoid the difficulties encountered with anomalous behavior of sensing electrodes and to reduce the number of electrodes in the vessel.

Other automatic photometric end-point titrators have been described by Malmstadt and Gohrbandt (*295*) for thorium determination and by Bischoff and Desreux (*34*) for the turbidimetric determination of cellulose acetate.

Cruse (*84*) in a review of electrochemical analytical methods covers automatic titrations briefly. Juliard and van Cakenberghe (*231*) also discuss automatic titrations in a general way with a number of specific examples of what may be done. Robinson and Briggs (*364*) have patented a recording automatic titrator.

By far the greatest activity in automatic titrimetry has been in the use of more or less conventional automatic reagent addition (buret or syringe) with potentiometric detection of equivalence. Frediani (*139*) has described an automatic Karl Fischer titrator which differentiates true from fleeting dead-stop end points. Other dead-stop end-point circuits for use in automatic titrators have been published by Monand (*316*) in the microdetermination of sulfur by the Zimmerman method and by Morpain and Tranchant (*319*).

A redox method was used in a high precision semimicro automatic titrator built by Wise (*471*). He determined iron by using the double end point observed in titrating, with a suitable oxidant, the sample which had been treated with excess titanous sulfate. Sabine's continuous titration method (*375*) for cholinesterase determinations depends on alkalimetric titration of the acetic acid formed by the enzyme.

Other apparatus which have been described include differential-potentiometric methods by Takahashi *et al.* (*420, 421*) and Jenšovský (*229*). By using this technique the latter was able to determine the equivalence point exactly, even when the titration curve (potential *vs.* titrant volume) did not show a distinct inflection.

A thermometric device for automatic end-point detection, described by Linde *et al.* (*275*), consists of a thermistor, a recording potentiometer, and a constant-flow buret. Plots of temperature *vs.* time showed a distinct break at the end points of several titrations of strong and weak acids and bases. High-frequency end-point detection has been used by Blaedel and Malmstadt (*37*) and by Musha *et al.* (*328*).

At least two patents have been issued for automatic titration systems involving pumps. Sheen (*396*) describes one which maintains a desired chemical concentration in a solution by adding reagent through a duplex pump operated by a controller depending on a suitable concentration sensing element—e.g., pH electrode, photometer, conductivity meter, and viscometer. Hallikainen and Pompeo (*170*) patented a continuous electrometric titrator in which the liquid sample is delivered to the titrating vessel at a constant rate by a synchronous motor-driven pump. The titrant, on the other hand, is delivered by a pump driven by a variable-speed two-phase motor. The electromotive force from the electrodes in the vessel is amplified and caused to regulate the speed of the titrating motor. This motor also drives a direct current generator whose output partially feeds a recorder-controller and partially feeds back into the amplifier circuit to make the e.m.f.–motor speed relationship linear.

Denton and Grauel (*95*) have endeavored to speed up manual titrations in biological assay work by automatizing the tube rinsing and waste discharge operations in the procedure. Solenoid valves actuated by microswitches, when in contact with the inverted titration tube, cause distilled water to flow into the tube, rinsing the contents into the titration beaker. Upon completion of the titration manually, pressing another switch opens the solenoid valve and drains the titration beaker. This arrangement allowed a single operator to conduct 120 electrometric titrations per hour.

Ozone is determined in an apparatus described by Carbenay and Vassy (*59*), in which the air sample is admitted to a reaction chamber in which ozone reacts with potassium iodide to release iodine. The liberated iodine depolarizes a pair of platinum electrodes in a saline electrolyte. The resulting current activates a mechanism which records the volume of gas sample consumed, then also drains, rinses, and recharges the apparatus. Bowen and Regener (*42*) determined atmospheric ozone with a semiportable device which similarly passes the air sample through a potassium iodide–sodium thiosulfate solution. Complete consumption of the thiosulfate causes iodine liberation and the same cycle of operations as described above, the volume of the gas necessary to consume all of the thiosulfate being recorded for each cycle.

EMISSIMETRY

The automatic measurement of spectrographic emission line intensities has become accepted as a time-saving cost-reducing necessity in many metallurgical industries. Hasler's reviews of quantometry (*182–184*) describe instrument advances in this field, emphasizing the attainment of greater instrument reliability over long periods of time. New methods have been developed for commercial nickel alloys, stainless steels (low and high alloy), biological solutions, slags, and aluminum-, zinc-, copper-, and lead-base alloys.

Brown (*48*) describes application of the Quantometer to production control in a steel mill, while Orsag (*333*) covers applications in the aluminum industry. The latter also found the instrument useful in comparing different brands of graphites, as well as in studying the influence of the sparkgap and the capacity and self-inductance effects of the generator on the analysis. Brehm and Fassel (*45*) have described approaches to direct-reading spectrochemical analysis, while Williamson and Robinson (*467*) added a computer which determines the log of the ratio of two concentrations for use with multicomponent mixtures. Saunderson *et al.* (*382*) patented an instrument for internal-standard methods. Walker and Baker (*449*) used a symmetrical cathode-follower bridge circuit for direct spectrochemical analysis which provides line intensity ratios directly. Naish and Ramsden (*329*) reviewed direct-reading spectrographs with a classification based on the two intensity measurements used: integrating and averaging.

Developments in Europe have been described by Benussi and Caroli (*30*) with particular attention to the production control of special steels, by Giavino (*153*) who discussed some aspects in proper calibration, by Hans (*172*) who recommended an apparatus for determining silicon and manganese in open-hearth and electric-furnace steels, and by Mathieu (*302*) who described the Spectro-Lecteur.

Emission from flames can be measured continuously. Opler and Miller (*332*) modified a Beckman DU and added a recorder which plots emission intensity *vs.* time. They used it to record sodium ion concentration in the eluate of an ion exchange column. Šafir (*376*) also described the use of a flame photometer in continuous laboratory analysis and applied it to the determination of potassium and sodium in inorganic salt solutions. Holiday and Preedy (*198*) and King and Priestley (*244*) modified flame photometers to be direct-reading.

Emission of a fluorescence nature can sometimes be useful in the determination of the few materials which fluoresce sufficiently well. Brown and Marsh (*49*) described a device for automatic measurement of fluorescence on paper chromatograms, consisting of an attachment including a spacer, a scanning chamber, a strip-transporting mechanism, a photomultiplier tube and amplifier, and a strip-chart recorder. The monochromator was a commercial one employing a stabilized light source. Similarly, gaseous hydrogen fluoride may be measured (*73*) in the atmosphere by contact with a moving strip of filter paper impregnated with a fluorescent salt. The hydrogen fluoride concentration is proportional to the fluorescence measured photoelectrically.

Vacher and Lortie (*441*) found that the luminescent emission spectra of organic vapors excited by a high-frequency radio field had important advantages over excitation by line-frequency current. Broida and Moyer (*46*) determined mixtures of hydrogen and deuterium spectroscopically by using the 150-megacycle electrodeless discharge of the flowing gas. Photomultiplier detection of the intensity ratio was used, and the Balmer lines were scanned at 10 A. per minute.

INFRARED ABSORPTIOMETRY

Without a doubt the widest advances in automatic analysis during the past 3 years have been in the use of infrared absorption measuring devices. Many new applications have been described (*69*) and some instrumental refinements are evident. Walters (*451*) has reviewed the practical considerations which govern the installation of continuous infrared analyzers for plant streams (both liquids and gases). Munch (*326*) also reviewed situations where infrared analyzers can be helpful, emphasizing that the prospective user must be aware of the operating principles involved. Voter and Hare (*447*) debated the improved infrared spectrometers from the users' and manufacturers' viewpoints. Devine (*98*) discussed the principles, applications, and existing problems of continuous infrared analyzers.

A number of patents have been issued for infrared analyzers of various designs (*188, 211, 413, 466*). Hales (*167*) in a general study of double-beam systems in absorption spectrophotometry arrived at a design which avoids difficulties inherent in some double-beam systems where the two beams may arrive at the detector with slightly different wave lengths. Other new nondispersive and dispersive instrument designs have been advanced by Bartley (*22*), Herscher and Wright (*192*), Hollander *et al.* (*199*), Patterson (*341*), Savitzky and Bresky (*383*), Smith (*406*), Waters and Hartz (*453*), and Woodhull *et al.* (*476*).

Case histories of applications, with much reliable experience reported, are plentiful. Applications of a wide variety are described by Smith (*405*), including both liquids and gases, with details of sensitization, calibration, and sensitivities. Wall *et al.* (*450*) decided that at least 17 infrared process analyzers were necessary for the continuous control of the acrylonitrile process and reported that test runs before plant start-up reduced the incidence of trouble later. Washburn and Austin (*452*), discussing instrumentation problems in atmospheric analysis, call for better fractionating and concentration techniques to enable the wider use of infrared measurements.

The determination of carbon monoxide has been described for various ranges down to 0.001% (*226, 285, 393*). Carbon dioxide likewise can be measured by its infrared absorption (*253, 284, 454*), as can nitrous oxide (*284*). Berger (*31*) and Martin and Thomas (*300*) describe applications to butane fractionating systems. The latter authors report their experiences with a 3-year installation, including completely automatic sample handling, switching, and restandardization. Uhl (*438*) reviewed infrared analyzers for petroleum processing control. Hoyer (*208*) used a special flow cell in which fluid from a chromatographic tube can be analyzed directly, either intermittently or continuously, by infrared means. Karasek and Miller (*232*) determined 0 to 0.4% water in liquid sulfur dioxide, while Massa (*301*) determined gaseous sulfur dioxide (0.005 to 1%) in stack gases.

The course of organic reactions was followed by infrared by Gauthier (*150*). Examples given were hydrogenation of propiolic acid and the maleate-fumarate isomerization.

LIGHT ABSORPTIOMETRY

A wide variety of types of flow colorimeters, nephelometers, turbidimeters, etc., is now available commercially, and recent emphasis in this field has been on design modifications and instrument component improvements for special applications. Several general papers broadly cover some aspects of the subject. Stair (*412*) gives examples of uses of filter radiometry in all optical wave-length regions. Foy's review (*137*) of smoke measurement covers photoelectric smoke determinations, as does Holt's (*203*) study of dusts in industrial atmospheres.

Many types of apparatus have been described, including flow colorimeters by Bishop and White (*35*), Dijkstra and Sieswerda (*101*), Cooke (*78*), and Plymale (*352*). Smoke and dust meters are covered by Galadzhiĭ (*146*), Gucker (*161*), Ramser and Hickey (*357*), and Smith and Carlisle (*404*), while instruments for measuring liquid turbidities are described by Formmer (*135*), Scragg *et al.* (*388*), and Underwood and Doermann (*439*). A novel device for measuring the water vapor content of gases has been patented (*129*) and depends on adiabatic expansion of the sample gas, resulting in cloud formation due to condensation caused by the cooling effect. The light transmittance of the cloud is a function of the moisture content. The recording is directly in per cent water content.

The spectrokinetic study of rapid reactions is possible in a device described for biochemical use by Dalziel (*88*). The automatic determination of sugar in boiler-feed water is continuously carried out with a sensitivity of 0.001% sugar in another apparatus (*143*) using a 1-naphthol reagent. Portable earpiece oximeters for oxygen in blood have been newly described by Paul (*342*) and Stott (*415*).

Gases may be determined absorptiometrically by bubbling into a liquid which develops a color dependent on the amount of the desired constituent. Thus, oxidants may be measured, such as ozone (*281*) by liberation of iodine from a potassium iodide solution, and chlorine (*255*) by reaction with *o*-tolidine, as well as hydrogen sulfide (*3*) by reaction with a $Na_2Fe(CN)_5NO$ solution, and carbon dioxide (*305*) in the range 0.06 to 12% by reaction with phenol red solutions. The latter device is operable indefinitely with operator attention to liquid level, temperature, and blank corrections only every 12 hours. Chlorine and chlorine dioxide concentrations are measured photometrically in devices described by Krumbein *et al.* (*256*) and by Murphy (*327*), respectively. Kiffer and Dowell (*243*), in attempting to measure ozone, found that ultraviolet absorption gave too erratic results, and they resorted to the visible region between 5600 and 6200 A., using a 48-inch tube to measure 0 to 10 mole %.

Light absorption of colored materials on filter papers used in chromatographic (*49, 323, 337*) or electrophoretic (*83, 264*) separations has been used by several authors.

ULTRAVIOLET AND X-RAY ABSORPTIOMETRY

Troy (*436*) described experience in the use of an improved highly sensitive portable photoelectric photometer for the direct measurement of ultraviolet-absorbing atmospheric pollutants, including chlorinated ethylenes, benzene, toluene, nitrobenzene, acetone, carbon disulfide, and dimethylformamide. The instrument is capable of reliable operation at full scale absorbances between 0.005 and 0.05. A calibrating accessory to minimize the adverse effects of adsorption can supply a continuously flowing standard gas sample at a rate of 500 ml. per minute for periods up to 30 minutes at concentrations between 0.2 p.p.m. to 20% by volume for gases in air or 0.1 p.p.m. to saturation for vapors in air. Campbell and Godin (*58*) and Kaye *et al.* (*237*) described automatizations of Beckman instruments.

Ultraviolet absorption of chromatographically separated substances on filter paper (*181*) has been accomplished automatically. Deutsch *et al.* (*96*) measured the ultraviolet absorbance of eluates from a chromatographic column. The spectrophotometer used was fitted for periodic standardization and automatic recording in studies of nucleic acid derivatives.

X-ray absorptiometry and diffractometry are the subjects of several "automatists." Roop (*367*) measured the total solids in liquid foods continuously by this method. Kistiakowsky (*246*) passed soft x-rays through gases to obtain time records of the gas density of a detonation wave. A scintillation counter with oscilloscopic indication measured the intensity of the transmitted rays. Pepinsky (*346*) discussed the feasibility of automatic crystal-structure analysis, and Stenhagen (*414*) described an x-ray camera for the continuous recording of diffraction pattern-temperature diagrams. The pattern falls through a horizontal slit onto a photographic film which is rotated at a speed proportional to the increasing temperature of the sample.

THERMOCONDUCTOMETRY

The time-honored method of gas analysis based on the thermal conductivity of some constituent(s) of the sample has been put to further use in a variety of novel approaches. The determination of ortho-para hydrogen is described by Grilly (*160*), who connected a pair of Pirani gage tubes in a bridge circuit so that, at liquid nitrogen temperatures, the bridge is balanced when both tubes contain normal hydrogen (25% para, 75% ortho) at reduced pressures. When the sample tube has a different composition in it, the resulting difference in thermal conductivity causes a change in the Pirani gage filament resistance and hence bridge unbalance.

The influence of an inhomogeneous electric field on the heat transfer of gases was applied by Senftleben and Gladisch (*392*) to analyze mixtures of polar and nonpolar compounds. Calibration curves were presented for propane–carbon dioxide mixtures, and some calibration curves can be predicted theoretically for such mixtures as ethyl chloride and acetylene.

The indirect automatic analysis of commercial oxygen is the subject of a German patent (*151*) which describes an apparatus based on the absorption of the oxygen by a liquid absorbent, and addition of a measured amount of hydrogen, followed by a measurement of the thermal conductivity. Purities of commercial oxygen (98.5 to 99.8%) were determined within 0.05%. Naumann (*330*) automatically analyzed waste gases for oxygen thermoconductometrically, with the aid of an inhomogeneous magnetic field (see Magnetimetry). Griffiths *et al.* (*159*) used thermal measurements to follow chromatographic separations of gases.

The measurement of moisture in a porous medium such as sand is described by Kawada and Uchida (*234*), who devised an instrument having two hot wires with different resistances forming two branches of a Wheatstone bridge. The amount of moisture in the sand affects the outward heat transfer of the wire buried in the sand. It was found suitable for water contents below 10%.

More or less traditional thermoconductometric instruments for plant control are described by Angerhofer and Dewey (*8*) for the production of oxygen, nitrogen, and rare gases from air, by Zaikowsky (*480*) for analyzing internal combustion gases to indicate fuel richness, by Richardson (*360*) for the determination of hydrogen and carbon dioxide, and by Pritchard (*354*) for measuring methane in air or carbon dioxide. Pritchard's device makes use of a convective instead of conductive heat transfer from the wire filament to insensitize the measurement to a third gas component. Hence, methane was determined in nitrogen-oxygen–carbon dioxide mixtures.

REFRACTOMETRY

Svensson (*418*) has reviewed the problems inherent in the refractometry of flowing solutions and defined the conditions under which errors become significant. Perhaps one of the more useful laboratory uses of continuous refractometry is in the monitoring of effluents from chromatographic columns. A variety of designs have been described recently. Kegeles and Sober's (*213, 238*) instrument used a cell-focusing cylindrical-lens optical system together with a vertically driven photographic plate, combined with an interferometer and an automatically recording prism cell. McCormick's (*289*) inexpensive device utilized razor blades for the slit and had an inner sample cell surrounded by an outer solvent cell. He found it useful for studying the chromatographic separation of silicone oils from a polyester solution. Seiflow (*390*) described a simple recording differential refractometer designed especially for chromatographic effluents containing radioactive materials. Trenner *et al.* (*434*) described a precision recording refractometer which plots mass or volume, as measured by a strain-gage circuit, against refractive index obtained in a flowing differential-type cell.

Ashman *et al.* (*12*) found a focusing cell refractometer useful in the manufacture of synthetic rubbers, where the monitoring of various hydrocarbon feed streams had to be continuous. Eltenton's (*123*) refractometer was also designed for following hydrocarbon reactions. Campbell *et al.* (*57*) described a recording differential refractometer for continuous plant stream monitoring.

Ingelstam (*214*) described a phase contrast refractometer for gases and liquids and gave examples of measurements on oxygen. Clamann (*76*) described a pneumatic refractometer to be used as a gas analyzer. The sample gas mixture was forced by constant flow to form a well-defined band embedded in a suitable reference gas. The light beam passing from the reference gas into the sample gas was refracted and the brightness of its image on a photocell was amplified and recorded.

REFLECTOMETRY

The continuing utility of measuring color changes of specially treated filter paper tapes when exposed to flowing gas samples is apparent in several references. Three patents cover such a technique applied to the determination of chlorine (*417*), hydrogen sulfide (*79*), and contaminants in exhaust gas (*446*). Gaseous hydrogen fluoride can be detected by the use of a roll of filter paper impregnated with a fluorescent salt (*65, 71*).

Two papers describe the utility of reflection measurement in the estimation of proteins separated by paper-strip electrophoresis (*83, 263*).

Lukens *et al.* (*286*) found a reliable correlation of the moisture content of yellow sweet corn with the reflected light measured by a specially designed recording filter photometer. Glasser and

Troy (*154*) described a new high-sensitivity differential colorimeter which can measure CIE tristimulus values and their ratios for sample to standard, to four significant figures for most colors.

CONDUCTOMETRY

Many applications of electrical conductivity measurements have been newly described, particularly for the determination of ionized materials in water and for the estimation of water in solids and gases. Several rather general reviews on the subject are worth noting (*118, 368, 371, 372, 456*).

Dissolved solids in water can be measured nonspecifically by conductance techniques, and new applications have been described for general monitoring of conductivity (*249*), deionized water (*70*), fluoride in water (*250*), salinity of sea water or brine (*180, 225, 290*), salt exchange in plants (*473*), monitoring the concentration of sodium hypochlorite baths (*28*), automatic titrations (*130*), and following the progress of chromatographic elutions (*109, 227*).

Vapors and gases can be measured by absorption in water or aqueous solutions (*372, 459*), including carbon dioxide (*64, 156, 221, 475*), hydrogen bromide (from methyl bromide thermally decomposed in hydrogen) (*165*), sulfur dioxide (*64*), and oxygen (*64*). Frequently the absorbing liquid contains a reagent which reacts with the desired constituent—e.g., chromous sulfate for oxygen (*64*). Sulfuric acid vapor in air is determined (*138*) in the range of a few parts per million by measuring the dew point increase as indicated by the electrical conductivity of a glass surface.

Water in gases has been successfully measured for several years by electrical hygrometers, and several improvements have been described (*2, 91, 120, 187*). Water in solids continues to be the object of study, particularly for moisture content of paper (*173*) and of soil (*41, 456*). Water in air has been also determined by passing the air through filter paper tape and measuring the conductivity of the paper (*94*).

Conductivity measurements of gaseous samples have been used to determine impurities. Kenty (*242*) patented a method involving gas samples at pressures of 0.1 to 10 mm. in a glass tube containing a small amount of mercury. When a low current is transmitted through the tube, a discharge occurs due to the impurity, and the resulting voltage drop is measured. Curves for nitrogen and carbon monoxide were given. Seitz (*391*) patented an apparatus for determining nitrogen in argon by an electric arc in the gas stream. Turnbull and Harrison (*437*) measured low concentrations of hydrogen in nitrogen, using a Pirani gage in a cell separated from the sample stream by a palladium membrane permeable only to the hydrogen.

POTENTIOMETRY

Automatic potentiometric measurement is today accepted as the most reliable convenient method of following changes in the pH of aqueous systems. General papers on this subject include several on waste treatment and color (*14, 118, 157, 185, 251*), and on control of cooking conditions, etc., in the paper industry (*349*). Buenger (*52*) has reviewed pH measurement, and new pH standards have been made available by the National Bureau of Standards (*216*).

Unique uses of pH measurement have been described by Jeffrey (*228*) in following chromatographic separations, and by Käuflein (*233*) in determining phosphine impurities in acetylene. Rovelstad *et al.* (*370*) reviewed factors concerning the continuous recording in situ pH of gastric and duodenal contents. They found greater accuracy when both the glass and the calomel electrodes were placed in the stomach. Fluctuations of 5 pH units in frequencies of up to 10 per minute were found.

Jacobson (*224*) and Fahnoe (*127*) described methods for determining oxygen in gases. Fahnoe's reduction potential instrument was adapted to the measurement of air in low pressure steam, while Jacobson's instrument is based on the depolarization by oxygen of a carbon cathode in a cell having a zinc anode. In the continuous recorder, the external circuit resistance is varied to keep the current constant, and the terminal voltage is thus proportional to the oxygen concentration. An electrolyte having a pH of 3 can be used to make the instrument relatively insensitive to carbon monoxide and carbon dioxide. A similar device is the subject of an Austrian patent (*462*).

A number of unusual applications of potentiometric measurements deserve mention. Russell and Cox (*373*) were able to determine directly ionic activity products, or ratios, in soils by using a series of electrodes, each sensitive to a different ion. Electrodes were made for calcium, manganese, aluminum, and zinc and, in soils not too acidic, magnesium. Examples of the type of information obtainable and the conditions under which the measurements can be made were given. Kwart and Wilson (*258*) measured reaction rates by using a high-speed recording potentiometer together with a stable high-impedance electronic detector and a glass electrode. They obtained solvolytic rate constants for *tert*-butyl chloride.

Phillips (*351*) devised an electronic method of detecting impurities in air, involving changes in the surface potential of specially prepared plates, as measured by a vibrating condenser technique and a selective phase-sensitive amplifier. A special metal reed vibrates above the surface being studied, and the alternating current voltage so developed measures the relative surface potential. The reed was steel, coated first with gold and then with calcium palmitate 20 molecules thick to give a stable potential. The plate was highly polished stainless steel specially cleaned by ionic bombardment. An example given was the determination of oxygen in ethylene intended for polyethylene production. In general Phillips found that 1 part in 10^7 of polar vapors could be detected. Heller *et al.* (*190*) used surface potentials to measure monomolecular films.

CURRENTIMETRY

The measurement of the magnitude of an electric current has been utilized in several important ways to automatize chemical analyses. Coulometric titrations, for example, have been described under Titrimetry, but several related techniques are not strictly titrations. Button and Davies (*54*) have described an apparatus for automatically recording the absorption of oxygen in a closed constant-volume gaseous system. Drops in pressure due to oxygen absorption are replenished with electrolytically generated oxygen, and the integrated electrolysis current provides a measure of the oxygen consumption.

Dissolved chlorine and oxygen are commonly measured amperometrically or polarographically, and new improvements continue to be developed. Clark (*77*) has reviewed amperometric techniques for chlorine residual. Haller's patent (*169*) describes an amperometric analyzer for determining concentrations of oxidizing or reducing gases, especially chlorine. The device consists of a tube, having an upper part of glass and a lower one of clay, filled with sodium chloride solution. The glass section has a silver electrode, while the clay section is wound with platinum wire. Leads from these electrodes are connected to a microammeter when the apparatus is immersed in a 2-liter beaker containing the gaseous samples, and the current indicates chlorine or chlorine dioxide (ClO_2).

Marks' patent (*299*) reveals a system for automatically determining the available chlorine in an aqueous system, where the aqueous potassium iodide is added to a flowing stream of the chlorinated water. After passage through a coil, to provide time for stoichiometric release of iodine, the mixture enters a depolarization type cell where the current indicates the available chlorine, whether present as $HOCl$, NH_2Cl, $NHCl_2$, or NCl_3.

Recording analyzers for oxygen in water are reviewed by Ippen *et al.* (*217, 218*), including the use of dropping mercury and rotating platinum electrodes. Freier *et al.* (*140*) devised an analyzer for oxygen in boiler water in the range of 6 to 70 × 10^{-6} gram per liter to ± 1 × 10^{-6} gram. Drenckhahn (*110*) has measured oxygen in blood, using a tiny cylindrical platinum electrode coated with collodion. Penneys (*344*) measured the oxygen tension of skin by the use of several electrodes.

Ion concentrations in flowing streams have been measured polarographically by Lewis and Overton (*273*). The apparatus consisted of two polarographs in opposition for measuring metallic ions having discrete well formed polarographic waves. Ishibashi and Fujinaga (*220*) described a differential polarograph having one dropping electrode, using a rotating switch connected in series with the electrode circuit. Optimum rotation of the switch caused two different voltages to be applied to the electrode and the corresponding currents could be recorded. Kemula (*241*) in chromatographic studies continuously monitored eluants from the column. Separations of cupric from cobaltous ions were nicely followed in this manner, and other possible applications were discussed.

A gas analyzer dependent on measuring the ionization current of a gas stream has been patented by Morgan (*317*). Quantitative results were obtained on essentially binary gaseous process streams. Brady and Zemany (*43*) studied the potassium ion emission from a hot platinum filament in the presence of various gases; oxygen, carbon tetrachloride, bromine, and helium cause a marked decrease in the potassium ion emission, while nitrogen has a smaller effect, and hydrogen causes an increase. The detection system was a modified Neir-type mass spectrometer with the focusing set for mass 39.

Previously reported potentialities of mass spectrometry for continuous analysis have been borne out by several developments. Miller *et al.* (*311*) used a portable mass spectrometer which recorded the concentration of five gases in a mixture every 20 seconds with 1% accuracy. The use of mass spectrometers in continuous process control has been described by Morgan *et al.* (*318*), Reinders (*358*), Robinson (*363*), and Walker *et al.* (*448*). Buckley *et al.* (*51*) described the use of a portable mass spectrometer for continuous alveolar gas analysis. Washburn and Austin (*452*) reviewed the uses of mass spectrometers in atmospheric analysis problems. Young *et al.* (*479*) described a mass spectrometer–digital computer combination for automatic analysis which resulted in completely automatic operation from the time the sample was introduced into the instrument until its composition appeared typed on a sheet of paper. An analysis of a mixture of 20 components was said to require less than 10 minutes from the time the sample was introduced.

DIELECTRIMETRY

New developments in the measurement of dielectric constants for moisture determination have been reported (*132*), for moisture in paper by Van den Akker and Hardacker (*442*), in fabrics by Sakagami *et al.* (*377*), in wet clay by Palmer (*336*), and in coking coals by Asbach (*11*).

Howe (*207*) and Uhl (*438*) discuss the general principles of dielectrimetric measurements for process control, and Thomas *et al.* (*425*) give a specific example in the continuous determination of toluene or total aromatics in petroleum process streams. Hashimoto and Mori (*181*) followed paper chromatographic separations with a high-frequency oscillator and found the technique applicable to all kinds of chemical substances, especially electrolytes. Gas chromatography, involving separations on charcoal columns, was the subject of work by Griffiths *et al.* (*159*), who measured displaced or eluted gases by various means including the dielectric constant.

Melville and coworkers (*53, 294*) have found the dielectrimetric method a useful one in following the nonstationary state in polymerizations. A special recording capacity bridge, having a sensitivity of 1 part in 10^8 with a time resolution of 10^{-3} second was used to measure the amounts of polymerization which occurred as a result of photochemical initiation. They have been able to evaluate the half times of kinetic chains by delineating the nonstationary behavior of certain polymerization reactions. They give dielectric data on styrene, vinyl acetate, methyl acrylate, and butyl acrylate. Using flash discharge tube illumination, they were able to study the decay of radical concentrations. Flom and Elving (*131*) used high-frequency oscillators to measure hydrolysis rates of esters. Current outputs were proportional to the ester concentration, and high-speed recording permitted the measurement of reactions with half lives of a few seconds.

RADIOACTIMETRY

Analytical uses of radioactive isotopes and radioactive measuring devices continue to multiply. Kohl (*252*) has reviewed radioisotopes in process control, and Parsegian (*339*) has described process instrumentation in the nuclear field. As with many other continuous measurement techniques, the utility of chromatographic separations has spurred the development of continuous radioactimetric monitoring. Devices for measuring activity *vs.* distance along a paper chromatogram have been described by Bonet-Maury (*38*), Frierson and Jones (*141*), Rockland *et al.* (*365*), Soloway *et al.* (*407*), and Winteringham *et al.* (*470*).

Jacobs and Lewis (*222*) discuss the use of a beta-ray instrument for determining hydrogen-carbon ratios in liquid hydrocarbons, and Kelly and Metzgar (*240*) discuss the automatic operation of a beta-ray spectrometer. Counters for measuring radioactive substances in air have been described by Baurmash and Dietrich (*23*), Labeyrie and Pellé (*259*), and Mansfield (*298*). Mansfield's continuous air monitor consists of two sampling assemblies, a Geiger-Müller counter for detecting beta-particles, a scintillation probe for alpha-particles, two counting-rate circuits, and recorders. The air sample is drawn through filter paper, which is then scanned by the detectors. Response times of 2 to 3 minutes and sensitivities of one tenth of the allowable concentrations of dusts are attainable, although not simultaneously.

Automatic counters featuring sample-changing devices have been available for several years, and new developments in this field are described by Anger (*7*), Bennett (*29*), Corbett and Honour (*80*), Harris and Del Duca (*177*), and Shirn and Wajda (*398*).

DATA

The analytical laboratory which does not now boast of several recorders and computing devices is an old-fashioned one indeed, and many new aids are becoming available to the analyst in collecting and handling the ever-increasing amounts of data he requires. Several reviews on recorders and plotting machines are of interest (*16, 385, 423*), and the use of punched cards has received renewed attention (*15, 63*). More specific devices have been described, such as the printing-recorder for use with scaling units described by Lang (*261*), the ordinate printing attachment for the GE recording spectrophotometer described by Miller (*312*), and the photoelectric measurement of the displacement of a luminous spot described by Thulin (*430*).

Other special attachments include Daly's (*87*) spectro-comparator for cathode-ray presentation of absorption-difference spectra, the device of Ramser and Hickey (*357*) to multiply light reflectance of a catalyst by gas flow to give instantaneous values of catalyst loss per unit time, and Walker and Baker's (*449*) cathode follower bridge circuit to give spectrographic emission line intensity ratios directly, with compensation for phototube dark current.

Anger's (*7*) counting-rate recorder plots points proportional to the arithmetic mean of the counts taken during fixed time intervals. Sperry's discussion (*408*) of process monitoring describes a system of automatic scanning equipment which warns of points off standard. Hogley (*197*) devised an instrument to measure and compute the mean and standard deviations of oscillations of a pen recorder arm. Müller (*321*) described a simple square-law computer with a circuit intended to develop potentials proportional to the square of the travel microscope used in studying the rates of diffusion of liquids through filter paper.

Weissler *et al.* (*457*) developed a graphical computer to transform a microphotometer trace of absorbance *vs.* wave length into an intensity trace. The instrument can take logs or antilogs of any variable graphically computed, as well as take the differences of two curves and plot the results either logarithmically or antilogarithmically. A computer described by Williamson and Robinson (*467*) also determines the log of the ratio of two concentrations and is especially applicable to direct reading spectrometers where the concentration of the internal standard cannot be considered constant.

MISCELLANEOUS

A number of automatic measurements do not fit conveniently into any of the above categories. Camp and Raymond (*56*) designed a photoelectric polarimeter for measuring transient rotations which uses two photomultiplier tubes to compare the intensity of an unanalyzed fraction of the light with a fraction which passes through a fixed analyzer. Rapid comparison of the output voltages of the two photocells yields a continuous record of the polarization of the incident beam and a rotation of 1 minute of arc can be detected.

Two instruments for sonic analysis are described by Wöhle (*474*) and Weller (*458*). Wöhle's device determines oxygen in the range of 20 to 40% with an accuracy within about 1%. Weller's acoustic apparatus records the air-fuel ratio in internal combustion engines. An oxidation furnace converts the exhaust gas to a binary mixture plus water vapor. Gucker's paper (*161*) on the determination of particulate matter describes both sonic and light-scattering techniques.

Innes (*215*) has described an apparatus for automatic adsorption, surface area, and pore volume measurement which depends on automatic adjustment of the gas pressure to a relative pressure of 0.2. This is done by passing the gas at a constant slow flow rate into the adsorption chamber and measuring the elapsed time.

Hawksley (*186*) described an improved method for automatically counting and sizing particles dispersed on microscope slides, based on measurements of the number of pulses occurring in the area swept by a scanning aperture. An automatic drop timer for dropping mercury electrodes has been described by Meites and Sturtevant (*310*).

Continuous electron-diffraction recording of chemical or physical transformations has been achieved by Trillat and Takahashi (*435*). They modified an electron-diffraction apparatus to obtain a photographic record of structural changes occurring with time, temperature, or other factors.

Microwave studies (*72, 210*) have shown potentialities for the use of this technique in following rapid reactions of gases.

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Natural and Synthetic Rubbers

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THIS is the sixth of a series of review articles on physical and chemical test methods pertaining to natural and synthetic rubbers. The first five appeared annually, but the present one is the beginning of a biennial series. The first one (*18*) covered only chemical methods up through the year 1948, but the second (*14*), third (*15*), fourth (*16*), and fifth (*17*) included physical testing.

The present review likewise covers both physical and chemical methods, and refers to articles appearing in the journals for the 2-year period ending October 1, 1954. Like the previous reviews, it omits test methods applied to compounding ingredients, except for their identification or quantitative determination in rubber. It also omits tests on raw materials used in the manufacture of synthetic rubbers. It does not refer to procedures which are concerned more with problems of a fundamental nature than with testing, unless they employ unique apparatus or techniques that seem likely to prove of value in testing procedures for rubber. Old procedures employed during this review period for either research or testing are not referred to again unless they have been modified or improved. Because of the extremely large number of articles on the subject of rubber testing published during these past 2 years, it has not been possible to make the survey exhaustively complete, but it is hoped that all the more important articles have been included.

GENERAL INFORMATION

During the past 2 years several review articles and books have presented general information on rubber testing. The most important sources of reference are the British monthly journal *Rubber Abstracts* and the annual reports for 1952 (*54*) and 1953 (*55*) on the progress of rubber technology, which are issued by the Institution of the Rubber Industry. Fisher (*65, 66*) has continued his annual review on rubber science and technology, in which he makes some reference to methods of testing rubber. Gehman's paper (*69*) on developments in the physics of rubber includes several new types of tests, such as the use of ultrasonic waves for tire inspection, and the use of sulfur-35 for determining the quantity of combined sulfur in a rubber compound. The Division of Rubber Chemistry of the American Chemical Society, under the editorship of Lerner (*126*), released the eighth volume of the "Bibliography of Rubber Literature," which covers the 3-year period 1946–1948. The editors expect to prepare these 3-year volumes each year until they are current.

Kreuter (*115*) continued his series of review articles, which began in early 1950, on testing methods used in the rubber industry. In another article on recent developments Braber (*25*) discussed the advantages and limiting conditions of laboratory testing as an indication of the practical behavior of rubber products. In a critical survey of rubber testing methods Millane (*144*), explained the importance of conducting tests over wide ranges of temperature, time, and stress. Several papers presented at the Third Rubber Technology Conference held in London in 1954 were related to testing methods. Good reviews of this conference were given in the rubber trade journals (*127, 149, 184*). Preprints of the papers have been made available, and the complete proceedings will appear later.

Committee D-11 (on Rubber and Rubberlike Materials) of the American Society for Testing Materials issued a revised "Standards on Rubber Products" (*2*) and a supplement (*3*) to the "ASTM Standards, 1952, Part 6." In these editions many of the older tests on rubber products have been revised and some new ones adopted. The British Standards Institution produced new issues of Part 2 ("Methods of Chemical Analysis") (*29*) and Part 4 ("Evaluation of Vulcanizing Characteristics") (*30*) of its Specification 1673 on "Methods of Testing Raw Rubber and Unvulcanized Compounded Rubber." Brazier (*27*) and Buist (*34*) reviewed the progress made in recent years in the standardization of rubber goods and of test methods, with special reference to the work of the British Standards Institution and similar standardizing organizations. Technical Committee TC 45 (Rubber) of the International Organization for Standardization held a meeting in June 1953, which has been reported in several places (*33, 51, 95, 195*). A number of the specifications for GR-S and GR-I synthetic rubbers made in the United States have been revised by the Reconstruction Finance Corp. (*173*).

Several reference books on rubber have been published during the past 2 years. The most recent is "Synthetic Rubber," edited by Whitby, Davis, and Dunbrook (*241*), which contains an excellent chapter by Juve (*97*) on "Physical Test Methods and Polymer Evaluation." Unfortunately, this book does not contain a similar chapter on chemical test methods. However, Wood (*247*) in his chapter on "Physical Chemistry of Rubbers" includes various testing procedures such as the quantitative determination of bound styrene in GR-S synthetic rubber. Howland's chapter (*91*) on "GR-S Latex" describes some tests used in the evaluation of the latex. Stern's recent book (*210*) on "Rubber: Natural and Synthetic" includes a chapter on the testing and analysis of rubber. Frey, in his book on methods of chemical analysis of rubber mixes (*68*), not only describes methods previously presented in the literature, but also adds his comments on methods with which he has had personal experience.

A loose-leaf binder book entitled "Technically Classified Rubber" has been issued by the Natural Rubber Bureau (*148*), which reprints information from various sources. Included in this book are the *News Sheets* and *Users' Information Circulars* on technically classified rubber, which are issued from time to time by the International Rubber Research Board. The American Society for Testing Materials has issued a special technical publication (*4*) which includes all the papers presented at a Symposium on Recent Developments in the Evaluation of Natural Rubber, held in New York in June 1952.

Noble revised and greatly enlarged his book, "Latex in Industry" (*158*), which was first issued in 1936 and has long been out of print. It contains all the procedures used in the chemical and physical testing of latex. The R. T. Vanderbilt Co., under the editorship of Winspear, has prepared a handbook on latex (*246*) which includes many latex-testing procedures.

Flory's "Principles of Polymer Chemistry" (*67*) does not pretend to be a textbook for the physical and chemical testing of rubber or latex, but it is such an excellent book that any technologist connected with the field of rubber should be familiar with it.

CHEMICAL ANALYSIS

The application of absorption spectroscopy to the analysis of rubber compounds has been described by Kendall and Davison (*103*), including the theory and the instrumental technique of both ultraviolet and infrared spectroscopy. The ultraviolet is most useful for estimating accelerators or antioxidants in rubber or combined styrene in a copolymer, while the infrared can be used for polymer analysis, as atomic groupings can be identified *in situ* in the polymer when prepared either in film form or in solution. A later paper (*47*) emphasizes the general identification and estimation of a wide range of elastomers by infrared techniques. However, because polymers are usually not easy to identify by conventional infrared techniques, Harms (*78*) and Kruse and Wallace (*117, 118*) found that a preliminary pyrolysis aided in the identification. Only a few drops of the pyrolyzate are necessary either for direct examination or as a solution in carbon tetrachloride. Richardson and Sacher (*177*) used infrared examination to determine quantitatively the different types of addition which take place when isoprene is polymerized. Identification of polymers from their pyrolytic products has also been made by mass spectrometric analysis. Using this method, Zemany (*252*) requires only a few tenths of a milligram of sample for the analysis.

Using methods based on the thermal decomposition of a polymer in a stream of nitrogen over superheated carbon, followed by the iodometric titration of the carbon monoxide formed, Unterzaucher (*234*) was able to make precise determination of the oxygen content. Kirshenbaum and Streng (*108*) compared the method of Unterzaucher with that of Grosse and Kirshenbaum (*74*), which makes use of isotopic principles, and found that the latter method yielded more precise results.

Meeks and coworkers (*141*) improved the existing gravimetric methods for determining rubber hydrocarbon in rubber-bearing plants. Comminution of the plant is accomplished by crushing between corrugated and smooth rolls, so that all the plant cells are ruptured and rubber is completely extracted. The bromination procedure is then followed. For determining gutta or rubber hydrocarbon in plants, Doman (*53*) developed a nephelometric micromethod which he believes to be superior to the usual gravimetric procedures. Salomon and coworkers (*187*) improved an old method for determining the amount of rubber in asphalt. A prolonged extraction with higher boiling solvents dissolves out the rubber, which is then made to react with a large quantity of sulfur. The rubber content is then determined from a sulfur analysis of the purified ebonite formed. The Rubber Research Institute of Malaya (*186*) states that the only accurate method for determining the dry rubber content of natural latex is to weigh a representative sample, coagulate it, dry the coagulum under specific conditions, and weigh the dried coagulum.

Linnig and coworkers (*132, 133*) presented a scheme for the analysis of gross chemical constituents of uncompounded GR-S synthetic rubber by complete solution procedures using a single weighed sample. The whole analytical procedure is less time-consuming than the extraction and other specification procedures, but unfortunately cannot be used for vulcanized GR-S or for GR-S containing gel.

Koide, Kubota, and Kuroi (*111*) compared various methods for the determination of total sulfur in vulcanized rubber. They report that the potassium hydroxide–potassium nitrate fusion method and the sodium peroxide fusion method are the quickest in manipulation but are less precise. Although the nitric acid–bromine oxidation–alkali fusion method is more precise than the previous ones mentioned, it is still not so precise as the nitric acid–bromine oxidation-nonfusion method recommended by the ASTM. The latter method is also the easiest to manipulate.

Khoroshaya and Kovrigina (*106*) recommend a rapid method for the determination of free sulfur. Its basis is the conversion of the free sulfur in the rubber (by means of sodium sulfite) into sodium thiosulfate, the combination of the surplus sodium sulfite with formaldehyde, and the titration of the acid-neutralized mixture with iodine. The whole procedure can be completed in about 30 minutes.

Khoroshaya and others (*105*) recommend that for ash determination in rubber a crucible be used which has an enlarged bottom surface of heat-resistant stainless steel. This crucible can be brought rapidly to the combustion temperature and thus saves considerable time. The authors recommend the procedure as suitable for routine testing.

A detailed procedure is given by Kress (*113*) for the quantitative analysis of the ash in rubber with the Spectranal. A simultaneous determination is made of the relative concentrations of several elements normally found in the ash. An accuracy within 20% of the amount present is achieved, and the procedure is considered to be practical wherever speed and convenience are of greater importance than absolute accuracy. Stern and Hinson

(*211*) found that in the ashing of neoprene some of the zinc present is volatilized, and they recommend that for the destruction of the organic matter a wet-oxidation procedure be used, similar to that which is often employed for copper and manganese in rubber.

Committee D-11 of the American Society for Testing Materials (*2, 3*) adopted two official methods for determining the "harmful" dirt in rubber, which is defined as that portion of the "insolubles" which does not pass through a 325-mesh screen. In method A, called the hot oil method, a large sample of rubber is mixed in hot oil and filtered hot through the screen. In method B, the solvent method, a small sample is dissolved in a solvent and the solution filtered through the screen. The former method is much more rapid, but unfortunately is much more difficult to manipulate. The methods are based on work done by a task group in the American Society for Testing Materials (*217*) and also by chemists at the Indonesian Rubber Research Institute (*84*). Heinisch (*82, 83*) later surveyed the problem of contamination of rubber by dirt and presented a critical discussion of the subject.

Wyatt (*249*) described in detail a method for separating small amounts of copper, manganese, and iron from each other and then determining their concentration by optical absorption measurements. Several other very good procedures (*12, 229, 233*) relate to the determination of minerals and other nonrubber constituents found in rubber and latex. Kidder (*107*) developed a method for determining the degree of yellow coloring in raw rubber latex films and crepes. The absorptions of the acetone extract of the rubber at wave lengths of 360, 400, and 560 mμ are combined by an empirical equation to give a measure of the coloring matter in the sample. The test is claimed to be effective even in the presence of considerable darkening due to the oxidation.

Rostler and White (*179*) have developed a procedure for the analysis of rubber extender oils in terms of asphaltenes, nitrogen bases, acidaffins, and paraffins, which can serve as a basis for standardizing the oils used in the oil-extended GR-S synthetic rubber. The Sun Oil Co. (*219*) suggests the classification of the oils as to viscosity index, or viscosity gravity constant, which is a useful measure of the nature of the oil in relation to its action in the rubber.

An exceptionally large amount of work seems to have been done these past 2 years on the identification and quantitative determination of rubber accelerators and antioxidants. Buscarons and Capitan (*37*) gave a series of comprehensive reviews of analytical procedures for 90 important accelerators known by their trade names, which they identified and classified according to chemical structure. Blokh, Kulberg, and Golubkova (*22*) described a method which can be completed in from 7 to 10 minutes for detecting thiuram and mercaptobenzothiazole in rubber mixes. Bauminger and Poulton (*11*) developed a colorimetric method for determining 2-mercaptobenzothiazole, which is based on the formation and evaluation of the red-brown color formed by the reaction of its alkaline salt with the nickel ion. The R. T. Vanderbilt Co. (*235*) described four reagents and the colors which they develop with the company's products.

Scheele and Gensch (*189*) described apparatus, procedures, and chemical reactions, and presented tables and graphs for conductometric titrations of dithiocarbamates, tetramethylthiuram disulfide and monosulfide, mercaptobenzothiazole, and diphenylguanadine. Hilton and Newell (*87*) developed a method for the quantitative determination of dithiocarbamates and thiuram disulfides in rubber. As the latter is converted into the former during cure, this method can measure the degree of conversion of the accelerator at any stage of cure.

Kress and Stevens (*114*) identified a number of curing agents in rubber products by ultraviolet absorptiometric analysis of selective solvent extracts. They claim their procedure to be more simple, specific, and sensitive than chromatographic procedures. Interference of the common softeners and antioxidants is usually negligible. Tanaka (*222, 223, 225*) made a spectrochemical study of 12 commercial organic accelerators in the ultraviolet before and after vulcanization in order to determine them and to estimate their proper distribution in rubber. The behavior of some of these accelerators was also studied (*224*) during various stages of vulcanization.

Brock and Louth (*31*) separated 24 accelerators and 12 antioxidants as to types, and made subsequent identifications by physical measurements such as x-ray diffraction and ultraviolet absorption. Fink and coworkers (*64*) developed procedures for the separation of accelerators by elution chromatography. When mercaptobenzothiazole is separated from interfering substances such as antioxidants and tars, ultraviolet spectroscopy offers a sensitive and specific identification.

Van Bloois (*23, 24*) gave a popular description of the chromatographic separation of ingredients such as accelerators, antioxidants, and plasticizers from vulcanized rubber. Parker and Barriman (*161, 162*) presented a scheme for chromatographic identification of 32 accelerators and antioxidants. The acetone-extracted material is chromatographed on silica gel–Celite in columns using a series of different binary solvent mixtures as developers. When the extruded columns have been streaked with the appropriate reagents, the positions of the zones, together with the colors of the streaks, give a reasonably good identification. Later these same authors (*163*) extended the work to include the mutual separation and identification of six alkyl-substituted dithiocarbamates.

Hamazaki (*77*) found it possible to identify several accelerators and antioxidants by ultraviolet observations. He observed the color change during development of the absorption band and the color reaction of cobalt oleate. Isayama (*96*) found the identification by copper sulfate solution more effective than by the cobalt oleate. Weber (*239*) developed a rapid and reliable technique for detecting accelerators by means of paper chromatography. Quantitative estimations can also be made by this method.

Hively and coworkers (*88*) found no interference from stearic acid, sulfur, paraffin, or rubber extract when they identified antioxidants by ultraviolet spectroscopic techniques after separation from one another by elution chromatography.

AGING AND DETERIORATION

Shelton, Wherley, and Cox (*199*) suggested that accelerated aging at only one temperature cannot predict accurately the aging characteristics of a stock, because at lower temperatures a cross linking of the molecules predominates while at the higher temperatures a chain-scission reaction is more likely to take place. Juve and Schoch (*98*) stated that an extrapolation of accelerated aging test data to room temperature requires a knowledge of the temperature dependence of the deterioration for the particular material being considered. Youmans and Maassen (*250*) tried to correlate room-temperature shelf aging with (1) individual test tube aging at 70° C., (2) oxygen bomb aging at 70° C. and 300 pounds per square inch and (3) air bomb aging at 127° C. and 80 pounds per square inch. None of these tests deteriorated all physical properties at the same rate, but the 70° test tube method most closely simulated natural aging.

Pollack (*168*) believes that the oxygen-absorption test provides a more fundamental and rapid evaluation of aging than do the other types of test. The oven test gives undue weight to aftervulcanization effects. The oxygen-bomb method does not produce a constant rate of loss of physical properties during the first few days; however, its 4- to 14-day rate should provide a good evaluation of the rubber tested.

Kirshenbaum, Streng, and Nellen (*110*) applied the isotopic method (*74*) of determining oxygen to the aging of rubber. This method would probably yield results similar to those obtained by measuring the oxygen absorption.

Heinisch's work (*80, 81*) with an improved "rubberscope" which heats natural rubber to 130° to 140° C. by infrared radiation shows that it is possible to determine within 20 minutes the aging behavior of rubber during storage. When this instrument is used in conjunction with plasticity measurements by the Hoekstra steam plastometer, it is especially useful in determining whether or not an excess of sulfuric acid has been used as a coagulant or an excess of copper or manganese is present in the rubber.

Zuev and Kuz'minskiĭ (*253*) found a close correlation between heat aging and light aging. The Institut Français du Caoutchouc (*94*) conducted some experiments on the relative influence of different wave lengths of radiation on the aging of rubber, and designated a specific high-pressure mercury vapor lamp to be used in the test apparatus to simulate the action of sunlight.

Work from two different laboratories (*99, 231*) has shown that materials can migrate from one rubber sample to another in the same aging oven, and thus change the aging characteristics of the samples. Contamination may also take place from one set of samples to the next set in the same oven, if the air in the oven has not been exhausted in the meantime. An apparatus is described for converting existing air-circulating laboratory ovens for isolated aging of eight or more different compounds.

Leigh-Dugmore (*125*) gave a critical historical survey of previous investigations into the causes of ozone cracking of rubber. Two distinct phenomena exist: the formation of cracks, and the subsequent growth of the cracks. He also discussed the methods used for measuring the length and depth of cracks.

Meynard and Madelaine (*143*) described apparatus and tests for assessing the effects of ozone on rubber mixes. They used a discharge ozonizer to produce the ozone, the concentration of which was determined by the iodometric method. Smith and Gough (*201*) studied ozone cracking by cinematography. They developed a technique of time-elapse photomicrography, from which was obtained a dynamic picture of the formation and growth of the cracks. Kalinsky and Werkenthin (*102*) applied radiometric procedures to the evaluation of surface cracking of elastomers. The volume of the crack is measured radiometrically from the volume of a radioactive powder included within the cracks. The use of radiations of different penetrability permits discrimination between volume and surface area of the cracks, the ratio of which is proportional to the average crack depth. Creed, Hill, and Breed (*42*) developed an apparatus for the accelerated evaluation of ozone-protective agents.

Crabtree and Biggs (*41*) studied the cracking of stressed rubber and found that it was caused not only by ozone but also by free radicals which could be formed by the photolysis of peroxides in the rubber.

Cooperative tests from nine different laboratories showed poor agreement in results of tests of flex cracking and crack growth on the DeMattia machine (*35*). Some changes in the present ASTM procedures are therefore recommended.

Hall, Conant, and Liska (*76*) developed a satisfactory apparatus and method for testing the long-time aging of elastomers under load. It more closely approximates service conditions than do most of the aging tests that have been reported.

Schoon (*192*) described a method for determining the growth rate of mold in raw natural rubber as a function of time. The samples are kept at 98% relative humidity, and the area covered with mold is measured every 24 hours.

Rogers and Heineman (*173*) reported that the conventional methods used for determining the aging characteristics of foam rubber offer little correlation and are not a true measure of the service life of the product. However, an oxygen-absorption test and a compression change aging test at 100° C. appear to give satisfactory evaluation of the aging of foam rubber in a relatively short period of time.

LATEX

Latex testing methods were the subject of an article by van den Tempel and Sinn (*230*), in which they attempted to correlate laboratory tests with the quality of the latex. The British Standards Institution (*28*) in 1954 issued a revised edition of its booklet on the chemical and physical testing of rubber latex.

The soap titration method for determining the average particle size of synthetic latexes has been modified by Cockbain (*38*) so as to make it applicable to natural rubber latexes. The method consists essentially of determining the distribution of an added detergent between the rubber and the aqueous phases at pH 6.0, the total detergent concentration being just sufficient for the formation of micelles in the aqueous phase. Ogilby (*159*) determined the latex particle size of both natural and synthetic rubbers by making titrations conductometrically with electrolytes at pH levels low enough to inactivate the soap stabilizers. He also used electron microscopic techniques, as did van den Tempel (*228*), for measuring the particle size distribution. Nisonoff, Messer, and Howland (*157*) used a method based on Stokes' law, which involves the use of an ordinary laboratory centrifuge. The method was shown to be valid by comparing the results with those obtained with light and electron microscopy.

In testing the mechanical stability of natural rubber latex by the usual procedure, the rod-dipping method for determining the end point has not proved very satisfactory. In order to improve on the method Meyer (*142*) made successive dips into the latex with narrow 80-mesh Monel screens, which were subsequently removed and washed free of adhering latex. The end point shows a rapid increase of adhering material. Because the usual mechanical stability tests for natural rubber latex are not suitable for the more stable synthetic latexes, Maron and Ulevitch (*137*) developed a test which subjects the latex to a shearing force exerted by a metal disk under load rotating in contact with a polyethylene surface. The amount of coagulum formed in a given period of time is taken as a measure of the stability.

Broese (*32*) described the nature of thixotropy and gave detailed instructions for measuring it, making use of a Couette rotation viscometer. Active fillers can be distinguished from inactive ones, but the ammonia content partially evens out the differences between latexes. Philpott and Sekar (*166*) described a method for measuring the volatile fatty acids in natural rubber latex. Resing (*175*) devised a chromatographic technique for studying the undesirable colored pigments in natural rubber latex.

Medalia, Townsend, and Grover (*140*) devised a unique method for measuring the wet gel strength of coagulant-dipped films. It employs an absorbent paper of low wet strength, impregnated with calcium nitrate, as a base for the deposition of a latex film. After the latex dipping the film is rinsed in water, dried, and tested on a tensile machine designed for high elongations and low tensile strengths.

UNVULCANIZED RUBBER

Under this general heading are discussed tests applied to unvulcanized rubber and also some tests on vulcanized rubber. Only the tests on vulcanized rubber which are used to determine the properties of the raw product or the characteristics of the process of vulcanization are considered.

VISCOSITY AND PLASTICITY

Scott (*194*) gave an account of a conference held by the British Society of Rheology. No single plastometer or viscometer can meet all the requirements of the rubber industry. In the rotation type the rate of shear is limited and the problems of slippage arise at the surfaces. The parallel plate type does not seem to be as suitable for fundamental studies.

In another article Scott (*196*) compared the rotation, extrusion, and compression types of tests, and concluded that for rapid plastometry the parallel plate compression test is the most suitable, provided that the test piece is precompressed to a small thickness for quicker heating to the test temperature before the load is applied. He also described the new Wallace modification of the Hoekstra instrument and discussed the fundamentals of all three types of instruments (*193*). The extrusion type does not measure true plasticity because of slippage between the rubber and the walls of the nozzle. The rotation type is the best suited for research work because of the good control of variables.

Whorlow (*242*) also compared the three types of instruments, and reported that there is no evidence of slippage in the Dillon extrusion plastometer and that this instrument may have advantages for testing carbon black–loaded stocks. The shearing-cone rotation viscometer is reported to be the best general-purpose instrument. Ecker (*57*) and coworkers (*58*) described the operations and gave references for the use of the Marzetti, Mooney, Williams, and Defo plastometers. Baader (*9*) made an evaluation and comparison of test results with the Defo and the Williams plastometers and concluded that the former has much better discriminating power for gradations of breakdown and is faster in operation.

In a study of the Mooney viscometer Decker and Roth (*49*) found that (1) viscosity readings decrease slightly as the depth of serrations of the rotor increases, (2) the serrations are unnecessary in testing commercial rubbers, (3) viscosity readings increase with an increase of either the thickness or diameter of rotor, (4) the usual rotor speed of 2 r.p.m. is too high to characterize high-molecular-weight polymers, and (5) the amount of surface slippage depends on the type of rubber and the type of metal surface of rotor and die.

Decker and Stiehler (*50*) showed further that in a Mooney viscometer an integral die and die holder transfers the heat much more rapidly than does the usual two-piece die arrangement. The integral unit therefore leads to a more uniform and better control of temperature conditions if the temperature is measured in the dies. Decker (*48*) also described a procedure for adjusting the Mooney viscometer die closures, which has led to considerable improvement in accuracy and reproducibility in die-closure adjustments. Methods of adjusting and operating the Mooney viscometer were also reviewed by Taylor and Veith (*227*), who discussed the effects of die closures, calibration, temperature, dimensions of parts, and sample treatment. Braun (*26*) stated that the velocity gradient in the plate and cone viscometers is not constant, as assumed by Piper and Scott, and he gave a formula to correct for this error.

Fensom (*62, 63*) found that the Mooney viscometer discloses much information on the structure of a high polymer. Evidence is shown of a relationship between the time interval from the start of shearing to the primary peak and the amount of free branching in the polymer.

Scott and Whorlow (*197*) showed that lack of precision in the preparation of plasticity test pieces for the parallel-plate compression-type plastometers will give variable results of measurement, and they described the technique of preparing test specimens for the Williams and the Defo instruments. Linhorst (*129*) designed a new compression plastometer which he claims can be adapted to the testing of relatively hard or soft materials by simply changing the temperature or pressure.

Bergen and Patterson (*20*) described a translational viscometer which can be used for measuring rubber latex. They found that latex of high solids content appears to be completely Newtonian, while the latex of synthetic rubber of 28% total solids content appears to be non-Newtonian.

VULCANIZATION

Stiehler and Roth (*216*) compared several methods recently proposed for the evaluation of the rate of cure of Hevea rubbers, and found essential agreement among (1) conclusions reached from strain and modulus data, (2) changes in Mooney viscosity during the initial period of vulcanization, and (3) measurements of stress and elongation at failure.

Veith (*236*) measured the state of cure of three classes of technically classified natural rubber by several methods which involve stress-strain testing and Mooney scorch testing. All methods placed the classes of rubber in the same order. The cure rate was the slowest for rubbers having lowest nonrubber content, and fastest for those having the highest nonrubber content.

Cretin (*43*) found a negative correlation between the vulcanization characteristic of a natural rubber, as measured by modulus test, and the dry rubber content of the latex; and a positive correlation between the modulus and the nitrogen content of the latex. These correlations are at least true if the latex is produced from the same general area and processed by a standard method.

Whorlow (*243*) showed that rotation and compression plastometers give equivalent data in evaluating cure characteristics, provided that allowance is made for any delay in the heating of test specimens used for the compression test. Sperberg (*206, 207*) found that the relaxed compression set test provides data which are highly indicative of a specific state of cure. Honda (*89*) determined the time of scorching and the velocity coefficient of scorching of rubber by measurements obtained with the Griffith extrusion plastometer.

Taylor, Clark, and Ball (*226*) found that the presence of moisture, even in very small amounts, has an effect on the rate of cure. Equivalent moisture contents may not have the same effect on all compounds, and thus the presence of moisture may make difficult the task of evaluating the curing characteristics of a rubber.

GRADING AND EVALUATION

Sears and Miller (*198*) of the Rubber Manufacturers' Association prepared a booklet which describes the RMA-type samples of crude natural rubber, and discusses purchase of natural rubber on the basis of visual inspection only. Vervloet and Noothout (*238*), however, recommend that the RMA types of rubber be classified further as to superclean, clean, and general purpose, depending on the amount of "harmful" dirt present in the rubber. LeBras (*124*) says that the producers know how to produce a more homogeneous rubber, but are not now doing so because their rubber would not fit into the present visual classification scheme of the association. The more recent technical classification scheme was therefore designed and adopted as an intermediate stage between the appearance and performance criteria as a basis for marketing rubber.

Several articles (*5, 13, 150, 151, 185, 251*) in the past 2 years have summarized the methods of technically classifying crude natural rubber and the progress which has been made. The International Rubber Research Board has continued its two series of publications, the *News Sheets* (*152, 153, 154*) and the *Users' Information Circulars* (*155, 156*), in which they keep rubber technicians informed on technically classified rubber. The three classifications based on Mooney viscosity were dropped, and the rubber is now graded only by its tensile properties (*153*). High-elongation modulus tests were found to be not as reproducible as low-elongation tests when compared under highly different climatic conditions (*153*), and modulus testing has since been shifting toward the lower elongations. Because of complaints from consumers that the properties of the classified rubbers too often fall outside the specification limits, these limits were changed so that now on the average no more than 1% of the rubber will be misclassified (*154*).

Vervloet (*237*) and Arentzen and Zeehuisen (*6*) report that interlaboratory variations in test results are very small in the tropics, even for unconditioned samples, but the results may be very different from those obtained in the cooler laboratories of Europe and the United States. These differences may actually cause a shift in the classification of the rubber. For this reason, it is advisable to have a standard natural rubber available in all testing laboratories, so that adjustments can be made in testing procedures which will place all the laboratories on an equivalent basis. McColm (*134*) showed that a standard uniform rubber could be made.

Wood (*248*) found large permanent increases in Mooney viscosity of the order of 30 or 40 units when freshly prepared raw natural rubber is stored under conditions of low relative humidity, especially below 30%.

VULCANIZATES

STATIC TENSION AND COMPRESSION

Gehman and Clifford (*70*) employed the Instron tester to explore new opportunities for the evaluation of rubber on the basis of stress-strain characteristics. The instrument was found to be excellent for research work, but not so adaptable to routine testing.

Cooper and Kersker (*40*) described a technique of preparing tension-testing samples of such high quality that there should be very little chance of low tensile results caused by faulty specimens. Labbe and Leeper (*121*) described a precise method for determining the width of a dumbbell test specimen used for tension testing. It requires the measurement of the thickness of the specimen by the usual type of gage, the weight of the sample, and the specific gravity of the rubber. According to Best (*21*), the molded strain test specimens used in the B.R.P.R.A. tester are likely to vary in cross-section area, and a simple method is given for correcting the resulting errors. Koide, Kubota, and Shinoda (*112*) recommend that in determining the "best" value for tensile strength the average of the four highest values be used when five specimens are tested, and when three specimens are tested the average of the two highest be used.

A simple device was described by Stafford (*208*) in which a steel spring of known characteristics is used as a "test piece" for calibrating the tensile testing machine. It enables a complete calibration under actual operating conditions. Stubbs (*218*) devised self-closing grips of the wedge type, which are used for holding rubber specimens in tension-testing instruments. Eller, Gondek, and Chatten (*60*) modified and improved their earlier machine for buffing vulcanized rubber specimens to uniform thickness before testing. It is claimed that rough strips of rubber can be buffed in this machine to a predetermined thickness with an over-all tolerance of 0.001 inch.

A recording stress relaxometer has been devised by McLoughlin (*135*), which automatically maintains the strain in the sample constant to within 0.001 inch during the complete relaxation of stress. Labbe and Phillips (*122*) developed an instrument for determining the rate of relaxation under compression or shear. It can also measure the recovery after partial or complete release of the load. The instrument can be used in the temperature range from −70° to 158° F.

From a mathematical examination of the influence of cross-sectional dimensions of test pieces of rubber used in testing for torsional deformation Gent (*73*) recommended that the test pieces be either square or circular in cross section if precise measurements are desired for the modulus of rigidity.

DYNAMIC PROPERTIES

The Goodrich flexometer was studied by Labbe (*120*), who found that the lack of reproducibility among laboratories is probably caused by the failure to adjust precisely the throw deflection of the test specimen. Actually, a change in load has less effect on the temperature rise and permanent set than any other single factor studied. Stechert (*209*) described a simple test for flexural rigidity. He also found that the test is sensitive to small differences in flexibility.

Davies (*45*) described a new testing machine which records autographic hysteresis loops and has an exceptionally broad frequency range. He found it very useful in studying the effect of frequency on the behavior of rubber under cyclic deformation. Davies (*46*) also pointed out that the two terms in current use for specifying hysteresis, "percentage damping" and "resilience," are complementary only if the hysteresis loop starts from zero strain. When a cycle is superimposed on a static strain, the terms are no longer complementary.

Kainradl and Haendler (*101*) classified fatigue tests, and stated that the rating of rubber compounds for heat build-up and fatigue life depends on the conditions of dynamic stressing, whether comparisons are made at constant amplitude or constant dynamic load. Dynamic measurements at room temperature are not adequate to predict heat buildup because of dependence of dynamic properties on temperature and amplitude.

Ecker (*56*) made a survey of instruments used for dynamic vibration testing, and described a new apparatus which enables measurements to be made of the plastic-elastic behavior of high polymers in the elastic range. Ourednik (*160*) designed an apparatus in which a cylindrical specimen is subjected to periodic compression, and the heat generated in the sample is measured. Gehman and Clifford (*71*) constructed a machine which they used to study the fatigue of rubber when subjected to biaxial strains of controlled amplitude and phase relationships. It was thought that this type of test would more nearly compare with service conditions than the relatively simple extension and retraction cycle usually used in laboratory testing.

A rotating drum camera was used by Muster and Volterra (*147*) for recording impact loading deformations. The camera records displacement-time data for short cylindrical specimens of rubberlike materials which are subjected to compressive impact loadings which last from 0.005 to 0.02 second.

HARDNESS

Kruse (*116*) and Royo (*180*) discussed problems in connection with the hardness testing of rubberlike materials and made tentative suggestions for finding a common basis for a uniform method of test which could be applicable to all types of materials.

Lichtman and Chatten (*128*) described a modification of the Admiralty rubber meter, which is particularly well adapted to precise measurements at low temperatures. A new bench-type hardness meter (*93, 181*) has been announced in which a dead load is applied to a spherical indentor by weights, not springs. An electric vibrator is built into the base to prevent friction and sticking in the machine. Several new meters (*1, 182, 183, 191, 221*) have been developed which measure the hardness of foam or sponge rubbers.

ABRASION

The abrasion properties of vulcanized rubber have been the subject of considerable review and discussion (*61, 100, 174, 188 190, 202, 203*). In general, the authors have dealt with the different types of testing machines; the comparison of their results with actual performance; the various factors which affect wear, such as temperature, coefficient of friction, and the hardness and elastic modulus of the rubber compound; the reproducibility of results; and the mechanism of abrasion. Kainradl (*100*) does not believe that a new type of abrasion instrument is needed, but that the present methods of test should be refined and better methods of interpreting their results found. Scheele and Hillmer (*190*) point out the necessity of making many more than the usual two to five individual abrasion tests on a particular compound in order to obtain a reliable average of the results.

Schallamach (*188*) developed an abrasion test in which he makes use of a needle bearing on a cylindrical test piece. The nature and direction of the abrasion patterns are examined microscopically and evaluated. Herzog and Burton (*85*) described a new machine which incorporates a rotating disk with an emery paper surface against which the rubber samples are abraded.

Powell and Gough (*169*) described in detail the modification of a constant-slip machine so as to make it conform with the constant-power principle. Baird and Svetlik (*10*) came to the conclusion that in order to correlate laboratory data with road tests the severity of service must be known.

Several investigators (*52, 92, 172, 204, 240, 244*) studied the wear on rubber and its relation to friction. Ratner (*172*) concluded that the index of wear depends on the load and is inversely proportional to the friction coefficient of rubber. He also stated that the coefficient of wear is constant for a given type of rubber. Denny (*52*) believes that at very low loads the friction is very nearly proportional to the load, while at very high loads the friction remains practically constant because the contact between the specimen and the track is complete. Wilkinson (*244*) described and illustrated a new method for evaluating the friction behavior of tire tread compounds.

ADHESION

Methods and conditions of adhesion testing were outlined and discussed critically by Swire and Hardy (*220*) and the common faults in the preparation of test specimens were shown.

Eller (*59*) described an inexpensive attachment to the Scott tensile tester which will measure the force required to separate a rubber strip from a metal base, the separating force being applied at right angles to the base. An autographic record can be made of the adhesive strength over the whole length of specimen. Pickup (*167*) developed a machine having a high natural frequency and appropriate damping in order to measure and record the fluctuations which take place in the usual constant-speed stripping tests.

LOW-TEMPERATURE TESTING

The literature on low-temperature test methods has been reviewed by Laurent (*123*), who discusses the testing for brittleness and for stiffness. Buist and Stafford (*36*) draw attention to the effects of continued exposures of elastomers to low temperatures over long periods of time. It must be remembered that crystallization is a function of time. Radi and Britt (*171*) made some low-temperature retraction studies of natural and synthetic rubbers over a wide range of temperature. They measured retraction at constant temperature, and plotted the percentage of retraction against the temperature at which retraction occurs. A deviation or dip in the curve from the sigmoid is an indication of the rate of crystallization at a specific temperature. It is believed to represent optimum thermal conditions for crystallization.

Labbe (*119*) studied hardness testing of elastomers at low temperatures using various instruments, and concluded that the spring-actuated instruments are desirable because they allow instantaneous readings. The Pusey and Jones and Olson instruments gave friction between moving parts at these low temperatures. It appears that the Materials Laboratory (modified British Admiralty) Indentometer is the most satisfactory of the dead-weight hardness testers for use at low temperatures. Morris and Barrett (*145*) find that hardness tests made on vulcanizates after conditioning for short periods of time at low temperatures are not always a true measure of the hardness which the vulcanizates will attain in the Arctic.

Sinclair and Griffis (*200*) describe the use of a modified Gehman torsional apparatus utilizing a liquid medium that measures accurately and rapidly the relative stiffening of rubber and rubber-like materials at low temperatures. Conant (*39*) finds that the different tests show good agreement in the temperature region of greatest technical importance, where the rubber is changing from the ordinary flexible state to a hard stiff material.

Marei (*136*) developed a procedure for obtaining the vitrification temperature of a rubberlike material by measuring the deformation of a polymer under compression. Belfer (*19*) found that a quick-acting wrench compression-set apparatus was superior at low temperatures to the screw-type apparatus for measuring short-time (10 seconds) compression set with respect to duplication of results, accuracy, and ease of manipulation.

TIRE TESTING

Williams and Clifton (*245*) gave a broad review of the newer techniques used in tire testing, in which they included indoor machine methods which attempt to simulate conditions on the road. Kern (*104*) stated that in order to have good abrasion properties the work of friction should be low, and he discussed techniques for measuring the work of friction in the evaluation of abrasion. Stiehler (*212, 214*) described new apparatus and methods used at the National Bureau of Standards for the testing of automobile and truck tires. Tests are made for (1) power loss and running temperature, (2) endurance or fatigue resistance, (3) energy of rupture (plunger test), (4) growth of tread cuts or cracks, and (5) tire life (road tests). A new machine is being developed which is designed to evaluate endurance, running temperature, cut growth, and tread wear in a single test. Stiehler, Richey, and Mandel (*215*) described a method for measuring the tread wear of commercial tires, in which the tires are weighed at intervals of wear. Reliable results are obtained from this method after 4000 to 6000 miles of operation. Their tests are designed statistically so as to compensate for inequalities of the wear of tires on different wheels. Comparisons of this weighing method are made with the method which measures the depth of groove. The former method will give reliable results with a small number of miles on the tire, while the latter method will not. Prat (*170*) compared Stiehler's method with two other methods and stated that all methods necessitate strict discipline if reliable results are to be obtained.

Morris, Stambaugh, and Gehman (*146*) gave a detailed description of an ultrasonic method of tire inspection. Internal flaws such as ply separation can be detected by measuring the attenuation of high-frequency sound transmitted through the tire. Horning (*90*) described and illustrated a portable instrument which measures tire temperatures within 2 to 3 seconds. The reading is claimed to be accurate to within 0.1° F., and the operator has control over the depth which the needle thermocouple is inserted into the tire. Axe (*8*) described some tests and the refrigeration methods used by the Dunlop Rubber Co. for low-temperature tire evaluation.

MISCELLANEOUS

A rapid method for measuring the specific gravity of rubber compounds, described by Gundavda (*75*), is claimed to be very good for routine measurements. It makes use of a tube of liquid with a density gradient. Such tubes are easily prepared, and the density gradient remains stable for many weeks. Linhorst (*130*) devised a new gravitometer which employs water and two springs for measuring the specific gravity of rubber compounds. Its speed and accuracy make it useful as a routine laboratory testing instrument.

Reynolds (*176*) constructed a special test cell using standard ASTM electrodes for measuring the dielectric strength of small specimens of elastomeric materials in air. The cell is constructed so that samples can be measured in a stretched or unstretched state. Pfestorf (*165*) makes use of conductivity measurements of rubber compounds to check the composition in the factory. The method is not only accurate for its purpose, but also very fast.

Patton and Hatfield (*79, 164*) made use of ultrasonic techniques to detect air films as faults in tires. Ultrasonics can also be used for measuring rubber thickness when only one side is available. Low frequency waves are used because of the high absorption of rubber and the scattering caused by the fabric. Heughan and Sproule (*86*) found that bond flaws and voids inside a flat-bonded specimen can also be detected by ultrasonic waves as long as the specimen is strained. Späth (*205*) also described the value of ultrasonic techniques in determining flaws in tires and in metal bonds.

Todd and Tramutt (*232*) developed a flame photometric determination for the rubber solids deposited on cords and fabrics. The method is based on following the sodium content of the system.

Czuha (*44*) described and illustrated a method for measuring the air permeability by diffusion tests, using a modified Warburg diffusion apparatus. Kirshenbaum, Streng, and Dunlap (*109*) described an isotopic procedure and apparatus for the rapid and accurate measurement of the permeability of gases through elastomers. They made use of radioactively labeled gases containing carbon-14. Auerbach and Gehman (*7*) employed radioactive sulfur-35 isotope in a new method for measuring the solubility and diffusivity of sulfur in rubber. By this method blooming characteristics may also be studied.

Geldof (*72*) presented an illustrated review and discussion of methods used for measuring physical properties important in evaluating the qualities of rubber soles, heels, and shoes.

Martin (*138*) studied the control of errors in physical test calculations in the rubber laboratory by introducing the statistical quality control system. The success of the method was shown by the gradual reduction of the incidence of errors over a period of time and a remarkable stability in the level from week to week. He also showed (*139*) how simple statistical quality control charts may be used to control the variability in results arising from mixing, compounding, vulcanizing, and testing.

Linnig, Mandel, and Peterson (*131*) devised a plan for studying the accuracy and precision of an analytical procedure. They illustrated their plan by the application of analytical tests on synthetic rubber. It involves the segregation of relative-type and constant-type systematic errors.

Stiehler (*213*) has shown that statistics has become a very important tool in the testing of rubber. It should be used in (1) planning and designing the experiment, (2) assessing the validity of data, (3) interpreting the experimental results, and (4) comparing the relative merits of the methods of test.

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Petroleum

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THE following review of advances in analysis in the field of petroleum covers the literature from the previous review (*105*) until approximately the middle of 1954.

CRUDE OIL

Karr, Weatherford, and Capell (*91*) demonstrated that undiluted crude oil can be chromatographically fractionated on activated alumina or activated bauxite, obtaining fractions which are sulfur concentrates, sulfur- and metal-free paraffins, paraffins-naphthene mixtures, aromatics, and an asphaltic fraction in which the vanadium content was concentrated twenty-fold. Pentane, benzene, and alcohol-benzene (1 to 3) were used as eluents. Putscher (*150*) separated olefins from Pennsylvania crude oil by silica gel adsorption and distillation, lending support to his previous finding that absorption at 10.3 microns is due to olefins which serve to characterize products from this crude oil. Adams and Richardson (*3*) employed efficient reduced pressure distillation to collect 61 fractions of a crude oil in which a trace of biphenyls was identified in two of the fractions by their radically different ultraviolet absorption spectra. Coons (*25*) removed metals from crude oil by filtering the reaction products of treatment with fluorosulfonic acid. Dunning, Moore, and Denekas (*35*) employed solvent extraction and chromatography to isolate porphyrins which were recognized by their absorption at 495, 530, 565, and 615 mμ. Stanfield (*167*) determined oil in lean oil shale by heating a 3-gram sample for 5 minutes at 600° C. Cuttitta (*28*), also employing a small sample of shale, determined oil potential from the absorption (420 mμ) of a toluene solution of its pyrolysis products.

GAS

Wirth (*191*) determined ethane, ethylene, and acetylene from the decrease in gas volume when the mixture was exposed to activated charcoal containing bromine in small excess; below −78° C. only ethylene reacted; at 0° C. acetylene reacted. Chawla (*21*) determined ethylene in the presence of acetylene by shaking with a solution of potassium iodomercurate to absorb the acetylene and then absorbed the ethylene in mercuric sulfate–sulfuric acid reagent. Brickell (*15*) determined moisture in natural gas by extraction at high pressure with ethylene glycol and titration of the solution with Fischer reagent.

Katz and Barr (*92*) determined olefins in hydrocarbon gas mixtures by a direct gas-with-gas titration technique, using pressure measurements to determine titer and end point with halogen gas reagents. Madley and Strickland-Constable (*120*) described an apparatus for analyzing as little as 20 cu. mm. of a gas mixture, employing solid absorbants held in stopcock bores. Legatski, Tooke, and Grundy (*103*) determined butanes and heavier alkanes in natural gas from the equilibrium pressure of 40 volumes of gas sample and 1 volume of *n*-octane at 37.8° C. Newton (*137*) extended the method of Hinden and Grosse to determine noncondensable impurities in condensable gases by mass spectrometry with neon as internal standard; the method can be used also to determine condensable impurities in noncondensable gases, where the choice of internal standard is wider. Klein and Struthers (*98*) determined naphthalene in coke oven gas by scrubbing with cyclohexane and measuring ultraviolet absorption of the solution at 309, 311.5, and 318.5 mμ, as little as 0.001% being determined. James and Phillips (*81*) described apparatus and method for analysis and separation of volatile substances by adsorption and partition chromatography. Charcoal was used in the adsorption column and dibutyl phthalate on Celite in the partition column. For liquids, a stream of nitrogen was bubbled through the sample and the saturated gas was then passed through the column. Adsorbed vapors were displaced by bromobenzene, higher boiling vapors displacing lower boiling ones to produce a series of peaks as the displaced components are detected by a thermal conductivity recorder. This method, which appears to have great possibilities, requires only a few centigrams of sample. Etienne (*43*) determined traces of propylene in gas by conversion to isopropyl alcohol using mercury-catalyzed sulfuric acid, oxidation of the alcohol to acetone by chromic acid, and condensation of the acetone with furfural to a red reaction product whose color is the basis of the determination. Baldwin and Daniel (*7*) described an apparatus for determining solubility of gases in gasoline and kerosine by saturating the liquid with the sample and subsequently determining the dissolved gas.

GASOLINE

Tilicheev and Okinshevich (*173*) determined the aromatic content of liquid fuels from the rise in freezing point of a cyclohexane solution of the sample upon treatment with concentrated sulfuric acid; less than 1 ml. of sample is required. Williams, Hastings, and Anderson (*190*) described an infrared method of analysis for determining individual alkyl benzenes through C_{10} and reported compositions of typical refinery aromatic stocks.

Norris and Coggeshall (*138*) determined classes of C_9 and C_{10} aromatics in gasoline distillation fractions by multicomponent ultraviolet absorption analysis based on the similarities of spectra of the individual members in each class of aromatics—namely, monosubstituted, ortho- and meta-substituted, and para-substituted. Only fair accuracy is claimed for the individual classes, but good accuracy was obtained for the total C_9 and C_{10} aromatic content. Interferences in thermally and catalytically cracked gasoline were first removed by treatment with alkaline permanganate. Bomstein (*14*) determined total aromatics in naphthas by measuring the area under the infrared spectrogram from 5.00 to 5.85 microns; he claims advantages over acid absorption, ultraviolet absorption, and Raman and mass spectrometry. Pasquinelli (*141*) determined aromatics in a wide variety of petroleum fractions boiling below 600° F. by the reduction in hydrocarbon volume that occurs when the sample is shaken for 2 minutes with a 22% solution of anhydrous picric acid in nitrobenzene. Harvey and Pearson (*65*), applying a method similar to that of Criddle and LeTourneau for determination of aromatics and olefins in petroleum distillates, found it advantageous to use bisdiphenylene-ethylene as silica gel adsorption indicator for aromatics with less than 9 carbon atoms and carotene for those of higher carbon content. Dienes blur the boundaries between hydrocarbon classes, but their interference was eliminated by refluxing the sample with maleic anhydride and eliminating the adduct before applying the chromatographic procedure. Shively and Morello (*158*) described a mass spectrometric method for determining most of the individual aromatic hydrocarbons from C_6 to C_9.

Polishuk, Donnell, and Wood (*145*) modified Rampton's dehydrogenation method (*152*) for determining cyclohexane-type naphthenes in petroleum naphtha to ensure a highly active catalyst; dearomatization was accomplished by acid absorption followed by silica gel percolation to eliminate sulfur contaminants before dehydrogenation. Friedel, Logar, and Shultz (*50*) determined hydrocarbon types in C_6 to C_8 fractions by mass spectrometry, subdividing the naphthenes into derivatives of cyclopentane and cyclohexane with subclasses of mono- and polysubstituted cyclopentanes and cyclohexanes. Sobcov (*164*) extended the mass spectrometric method for type analysis of gasoline and kerosine to determine ratios of normal paraffins and isoparaffins after preliminary distillation of the sample and preparation of a saturated portion by acid treating. The method is based on the difference in abundance ratio of fragment to parent ions for paraffins and isoparaffins. Lumpkin, Thomas, and Elliott (*114*) determined paraffin, naphthene, aromatic, and condensed-ring naphthene hydrocarbons in samples of low olefin content by a modification of Brown's mass spectrometric method for hydrocarbon-type analysis of naphthas. The method is based on the abundance of groups of peaks which characterize the compound types. Rampton (*152*) discussed the utility and limitations of methods for type analysis of hydrocarbon fuels. Melpolder, Brown, Young, and Headington (*127*) employed a combination of many modern techniques in analyzing a naphtha from fluid catalytic cracking and presented a very comprehensive composition analysis. Sobcov (*163*) concluded from a consideration of the application of matrix and IBM techniques to the analysis of a naphtha fraction (28° to 110° C.) that fractionation by distillation into at least four cuts is necessary and the largest useful matrix is of the fifteenth order.

Meyerson (*129*) determined benzene, cyclohexane, and methylcyclopentane in a C_6 distillation fraction by mass spectrometry from the peaks at mass number 78, 84, and 69, respectively. Hammer and Roe (*62*) described optimum conditions for determining cyclohexane in petroleum concentrates from the infrared spectrum at 11.0 to 12.0 microns. De Malde, Mussa, and Nebbia (*33*) determined traces of divinylacetylene in acrylic nitrile from the turbidity produced by its reaction with mercuric sulfate in dilute sulfuric acid and by its strong absorption in the infrared and ultraviolet.

Das (*30*) determined some ethylene compounds by reaction with mercuric acetate and nonaqueous titration of the excess reagent. Porter and Wood (*146*) showed that nitrogen tetroxide is not a satisfactory general reagent for the determination of olefins because the yields of crystalline reaction products are often low and sometimes oily messes are obtained instead of crystalline products. Liu and Chu (*110*) proposed a double-bond index, derived from refractive index, molecular weight, and hydrogen content of the sample before and after hydrogenation; they claim the method useful for identifying types of aromatic or olefinic hydrocarbons in petroleum fractions. Kazanskii, Rozengart, Sterligov, and Tarasova (*93*) proposed a new formula for calculating aromatic hydrocarbons from specific dispersion, as in their view existing formulas are unsatisfactory because of deviations from additivity of dispersion.

KEROSINE AND HEAVIER FUELS

Ward, Moore, and Ball (*180*) distinguished among straight-run, catalytically cracked, and thermally cracked fuels by the color produced upon shaking the sample with a mixture of phosphoric and acetic acids. Charlet, Lanneau, and Johnson (*20*) employed a combination of chromatographic, infrared, and ultraviolet absorption techniques in conjunction with molecular weight and carbon-hydrogen determination to determine polynuclear aromatic hydrocarbons in heavy gas oils. Hess and Arnold (*70*) separated naphthalene and more highly condensed systems from monocyclic and alkylated monocyclic aromatics in cycle gas oils by reaction with tetrahalophthalic anhydride to form a solid adduct. Eby, Priestley, Rehner, and Hall (*39*) proposed five physical-chemical methods for determining polycyclic aromatic hydrocarbons in high boiling petroleum products to provide nonbiological methods for predicting carcinogenic activity. These methods include ultraviolet absorption of a 343° to 538° C. cut, ultraviolet absorption of an aqueous caffeine extract of the sample, refractive index of an aromatic fraction isolated by adsorption on silica gel, and polarography. Cromwell, Rescorla, and Milsom (*27*) correlated drum storage stability of fuel oil with a laboratory oxidation test from which samples are periodically taken and insoluble gum is determined from the color of deposits on a pad through which the sample was vacuum filtered. McLaughlin and McClenahan (*118*) showed that *n*-paraffins containing 14 or more carbon atoms may adduct with thiourea, contrary to opinion that only branched paraffins will do so.

LUBRICATING OIL

Waterman and Booy (*181*) discussed graphical tracing of reactions of hydrocarbon mixtures and its relation to various ring concentrations and other ring properties. Martin and Sankin (*123*) presented a graph for calculating the average number of aromatic rings and the number of naphthene rings per molecule in aromatic hydrocarbon concentrates obtained by silica gel adsorption or solvent extraction. The determination of rings was based on density, specific dispersion, and molecular weight, the latter being derived from viscosities or density and midboiling point. Francis (*49*) employed infrared absorption for structural group analysis of high molecular weight saturated hydrocarbons; he reports methyl, paraffinic methylene, cyclopentyl, and cyclohexyl groups. Hazelwood (*67*) modified the method of Martin and Sankin for determining aromatics in petroleum concentrates by employing a new factor—density intercept—to permit use of refractive index instead of the more difficultly determined specific dispersion. Dunkel, Ford, and McAteer (*34*) employed chromatography to characterize petroleum oil extenders for synthetic rubber and to estimate asphaltene, nonaromatic, aromatic, and polar constituents.

Poti, Gent, Pomatti, and Levin (*147*) determined small amounts of a hindered phenol (4-methyl-2,6-di-*tert*-butylphenol) in unused and used lubricating oils from infrared absorption measurements at 11.63 microns before and after heating the sample to eliminate the additive. Woelfel, Good, and Neilson (*192*) determined traces of furfural in lubricating oil by extraction with aqueous sodium bisulfite, oxidation of the extract with iodine, and subsequent colorimetric determination of liberated furfural from its red reaction product with aniline.

Abe (*1*) described an apparatus for determining dielectric constant as a measure of water in oil. Stringer (*169*) described a humidiscope for determining water in oil. It depends on the pressure difference resulting from the diffusion of water vapor from the sample in one chamber to a desiccant in another. Meelheim and Roark (*126*) improved the method of Roberts and Levin for determining water in oil and grease by modifications in the distillation apparatus and substitution of toluene for benzene as azeotrope former.

Evans and Matthews (*44*) concluded from a study of methods for determining size of insoluble particles in used crankcase oils that results from direct measurement on photomicrograph, sedimentation under gravity, and centrifugal sedimentation are in good agreement. Clark, Mueller, and Culmer (*22*) found a definite relationship between optical properties (320 to 650 mμ) and chemical and physical data which in used lubricating oils indicate extent of oxidation and deterioration through use. They found that the slope of the optical curve is a good indication of the stability of the oil. Cookson, Coppock, and Schnurmann (*24*) determined the degree of refining of mineral oils which had been absorbed by bread in manufacture, from the ultraviolet absorption characteristics of the 85% sulfuric acid unsulfonatables of the total oils extracted from the bread by carbon tetrachloride. Jones and Milberger (*83*) employed thermal diffusion to separate high-boiling azeotropes and close-boiling components.

ASPHALT

Traxler and Schweyer (*174*) employed extraction with butyl alcohol and acetone to separate asphalts into asphaltic, paraffinic, and aromatic and naphthenic classes, claiming that methods producing more than three groups are difficult to interpret. Hubbard, Stanfield, and Kommes (*78*) found that the adsorption characteristics of alumina vary with source and method of preparation to such an extent that portions of the same sample of alumina should be employed when comparing constituent analyses of different asphalts. Higgins (*71*) determined the asphalt content of lubricating oil residua from the change in color of a naphtha solution of the sample upon treatment with an adsorbent clay; the decrease in color was proportional to the asphalt adsorbed. Traxler and Schweyer (*175*) determined the refractive index of asphalt by extrapolation from the refractive indices of naphtha solutions of the sample. Mariani (*122*) employed paper chromatography to recognize the presence of oils and resins in asphaltenes. Weaver and Gilkeson (*182*) developed an empirical relationship between penetration and viscosity and claimed it to be useful for control in asphalt manufacture.

SPECIALTIES

The identification of polymers from the characteristics of their pyrolysis products was accomplished with mass spectrometry by Zemany (*193*) and with infrared by Kruse and Wallace (*100*) and by Harms (*63*); the general subject was reviewed by Achhammer (*2*). The conditions of pyrolysis must be carefully followed in calibration and in analysis because the spectra of the pyrolysis products, although reproducible, are generally empirical. Meeks, Crook, Pardo, and Clark (*125*) determined rubber hydrocarbon in rubber-bearing plants by extracting with a benzene solution of trichloroacetic acid, brominating the solution, and weighing the precipitated rubber bromide. Kisfaludy (*97*) determined anthracene by reaction with maleic anhydride and dioxane at 125° C., back-titrating the excess of anhydride acidimetrically. The method of Criddle and LeTourneau for analysis of hydrocarbon mixtures by adsorption on silica gel was modified by Ellis and LeTourneau (*41*) for the determination of aromatics, saturates, total hydrocarbons, and total oxygenated compounds in lacquer thinners. Displacing agent was *n*-butylamine and rhodamine B base was added to the fluorescent indicator to mark the boundary between oxygenated compounds and the amine.

A countercurrent extraction method for the analysis of greases containing inorganic thickening agents was described by Coenen and Urner (*23*). Brunstrum and Grubb (*17*) found from a study of apparent viscosities obtained with both circular and rectangular capillaries that smooth greases gave similar viscosities, whereas fibrous greases gave lower viscosities with the rectangular capillary. They concluded that these differences can be used to recognize variations in the fibers of grease which are not evident visually. Mysels, Pomeroy, and Smith (*136*) found that ordinary acetone contained enough water to introduce a large error in the determination of free fatty acids in aluminum soap when this solvent is employed to extract the acids for titration; dry acetone greatly reduced the rate of hydrolysis of the soap. Hoyt and Walter (*77*) determined sulfonates in soaps by titration with specified cationic surface active agents, using bromophenol blue as a transition indicator, in a two-phase system of water and chloroform. A trace of excessive cationic agent was revealed by its deep-blue chloroform-soluble complex with the indicator; the sulfonate and cationic agent produced an insoluble complex. Gordon and Urner (*57*) determined inorganic salts in petroleum sulfonates by amperometric titration with lead nitrate.

Sweeney and Bultman (*171*) applied partition chromatography with water on silica acid in the separation and identification of tar acids and creosotes, effectively resolving mixtures of phenols.

POLLUTION

Mader, Heddon, Lofberg, and Koehler (*119*) employed a freeze-out technique to prepare a concentrate of the pollutants in the atmosphere after preliminary scrubbing to eliminate oxygenated compounds. Infrared absorption (3.45 microns) on the concentrate in a 100-cm. gas cell (rock salt windows) indicated a sensitivity of 0.1 p.p.m. in terms of hexane in the atmosphere. Senderikhina (*157*) determined hydrocarbons in the atmosphere by burning over a hot platinum spiral and claimed a sensitivity of 0.001%. Quiram, Metro, and Lewis (*151*) determined hydrocarbon contaminants in the atmosphere by adsorption on silica gel at −73° C. and desorption into a mass spectrometer for analysis; 95% of C_3, C_4, and C_5 hydrocarbons and 60% of ethane was recovered.

Kobayashi (*99*) determined benzene in air by passing the sample through a tube packed with silica gel containing sulfuric acid and formaldehyde. The length of discoloration was a measure of the benzene. Stitt and Tomimatsu (*168*) detected as little as 0.004 p.p.m. of ethylene in oxygen by oxidation with mercuric oxide. Selenium-sensitized paper strips were used comparatively to determine the evolved mercury equivalent to the ethylene. Patterson and Mellon (*142*) described the preparation of an active silica gel containing ammonium vanadate through which the sample is drawn. The resulting color intensity or linear discoloration formed the basis of the determination of sulfur dioxide. Sonnenschein and Schafer (*165*) determined less than 1 γ of hydrogen sulfide or carbon disulfide in air; they used a colorimetric procedure at 670 mμ for hydrogen sulfide after conversion to methylene blue by reaction with dimethyl-*p*-phenylenediamine and at 430 mμ for carbon disulfide after conversion to yellow cupric diethyldithiocarbamate.

In a study of atmospheric factors in smog formation Littman and Benoliel (*109*) developed a continuous recorder for oxidant in terms of ozone, which was based on liberation of iodine from a buffered neutral potassium iodide solution. McCabe (*116*) automatically measured the total oxidant in atmosphere by oxidation of phenolphthalin to phenolphthalein. The change from colorless to pink in alkaline solution is proportional to the oxidant.

Dangl and Nietsch (*29*) detected as little as 0.001 γ of petroleum in a liter of water by its fluorescence in ultraviolet light. Melpolder, Warfield, and Headington (*128*) determined as little as 10 parts per billion of gasoline in drinking water by stripping the boiling water with hydrogen, condensing the vapor at liquid nitrogen temperature, and analyzing the condensate by mass spectrometry. Carbon tetrachloride was used as carrier for the condensed hydrocarbons to render transfer to the mass spectrometer practical for concentrations above 0.01 p.p.m. The method can be used to detect kerosine and furnace oil in water by substituting *o*-dichlorobenzene for the carbon tetrachloride carrier. Levine, Mapes, and Roddy (*107*) determined the oil content of effluent waters from the change in density of carbon tetrachloride used to extract the sample. Herd (*69*) used paper strip chromatography in ultraviolet light to determine identity of oil taken from polluted navigable waters with the oil on a particular ship.

Wedgwood and Cooper (*184*) detected polynuclear hydrocarbons in gasworks and sewage effluents by percolating a cyclohexane solution of the hydrocarbon constituent of the sample through a column of alumina. A large number of fractions of eluate were collected and examined for ultraviolet absorption peaks characteristic of each hydrocarbon. Less than 1 mg. of hydrocarbon is sufficient sample. These authors (*185*) concluded that washings from macadam roads and the atmosphere also contained these constituents.

Headington (*68*) discussed the cooperative work of the American Petroleum Institute on six methods for determining oils or phenols in water. Schmauch and Grubb (*155*) determined phenols in waste water by extracting the sample at pH 12 with carbon tetrachloride to eliminate oil, then with tributylphosphate at pH 5 to concentrate the phenols and ultraviolet absorption at 3010 A. Spectrophotometric measurements at 3010 A. on a portion of the concentrate at pH 12 and another portion at pH 5 form the basis of the phenol determination by the bathochromic-difference spectrum.

ELEMENTS

Milner, Glass, Kirchner, and Yurick (*131*) described a wet-ashing technique for concentrating trace metals of crude oil. This method obviates losses of metals that may occur on dry ashing. The decomposition was done in a Vycor vessel with approximately half a volume of concentrated sulfuric acid; heating was from the top with an infrared lamp. Colorimetric methods are described for iron, nickel, and vanadium and a polarographic procedure is given for copper. Karchmer and Gunn (*88*) employed a very carefully controlled ashing technique in platinum at 538° to 566° C. to minimize the loss of trace metals which may occur at higher temperatures. Garner, Fellow, Green, and Harper (*52*) ignited at 450° C. for the same purpose and showed by comparison with wet oxidation by nitric acid and sulfuric acids that no appreciable loss of vanadium occurred. Dyroff, Hansen, and Hodgkins (*36*) compared spectrometric and semimicromethods for the determination of trace metals in ash from petroleum fractions and concluded that the precision of the two methods is similar; the spectrometric method has the advantage of speed.

Myasnikov (*134*) detected tetraethyllead in gasoline from qualitative tests for lead in the deposit left on the wick tip of a burner in which the gasoline was used. Levine (*106*) determined tetraethyllead in gasoline by the intensity of red color produced by dithizone and the hydrochloric acid extract of the sample. He claims the method is rapid and suitable for routine control. Preis (*148*) used an alcohol solution of iodine to determine tetraethyllead in aviation gasoline in a direct iodometric method claimed to require 5 minutes for a determination. Grunwald (*60*) determined tetraethyllead in gasoline by brominating and completed the determination by adding the sodium salt of ethylenediaminetetraacetic acid and titrating with zinc sulfate to Eriochrome Black T indicator. Gilbert (*55*) described a direct flame photometric method which is sensitive to 0.001 ml. of tetraethyllead per gallon. Jordan (*85*), in another flame photometric method, minimized linear relationship errors by employing as a standard a known of approximately the same lead content as the sample. Swanson and Daniels (*170*) applied polarographic and amperometric methods to aqueous acid extracts of lead in gasoline, lubricants, and deposits. They distinguished ionic lead from tetraethyllead by passing a butyl Cellosolve solution of the sample through a column of base exchange resin which retained the ionic lead.

Feigl and Caldas (*45*) detected as little as 0.025 γ of copper in gasoline by the blue complex it forms with alizarin blue. Livingstone and Lawson (*111*) determined as little as 0.025 mg. of copper per liter of gasoline by reaction with sodium diethyldithiocarbamate and measuring the density of the resultant yellow color at 440 mμ. They found that 0.1N hydrochloric acid was more effective in extracting copper from gasoline than 4N acid. Jordan (*86*) used flame photometry on the dilute hydrochloric acid extract of gasoline to determine copper, claiming less interference from lead and iron than in chemical methods. Buchwald and Wood (*19*) determined copper in lubricating oil by percolating an isopropyl alcohol–benzene solution of the sample through a column of ion exchange resin (hydrogen form of Zeo-Carb), eluting the copper with dilute sulfuric acid, and completing colorometrically with Versene. Barney (*8*) determined copper in turbine oil spectrometrically by dropwise ashing of 3 to 10 drops of the sample in graphite electrodes mounted in heating coils. A spectrographic method involving evaporation of the oil sample on a hot platform electrode was employed by Rozsa and Zeeb (*153*) who avoided ignition to minimize loss of metals.

Ham, Noar, and Reynolds (*61*) compared four spectrographic methods for the direct determination of metals in oils. Meeker and Pomatti (*124*) described a spectrographic method for determining traces of wear and corrosion metals in lubricating oil. The method involves direct current arc excitation of the residue left upon low temperature carbonization of a mixture of sample, barium matrix, nickel internal standard, and graphite powder. Barney and Kimball (*9*) determined traces of iron in used lubricating oil by ashing one drop of sample containing internal standard in the graphite cup of a lower electrode in which one drop of buffer had been evaporated. Pickles and Washbrook (*144*) determined zinc in lubricating oils by amperometric titration in a strongly basic solution containing cyclohexane, with aqueous Versene solution at the dropping mercury electrode. Moberg, Waithman, Ellis, and DuBois (*132*) determined calcium in lubricating oil by direct flame photometry. Davis and Van Nordstrand (*31*) employed a helium path to extend the applicability of x-ray fluorescence to determine barium and calcium, as well as zinc, in lubricating oil.

Hinkel and Raymond (*72*) described a modification of the Unterzaucher method for determining oxygen in organic substances. Their method involves passing into standard alkali the carbon dioxide resulting from the oxidation of carbon monoxide by iodine pentoxide and back-titrating the excess. Jones (*84*), reporting on the activities of an American Petroleum Institute committee, showed that 0.01 to 1.0% of oxygen in petroleum products can be determined by four different modifications of the Unterzaucher procedure. Modification was required to avoid error from the action of pyrolytic hydrogen on the iodine pentoxide. The modifications employed titrimetric, gravimetric, and manometric techniques. Oita and Conway (*139*) also modified the Unterzaucher method by employing as catalyst 50% of platinum on carbon instead of carbon alone; the change permits an operating temperature of 900° C. instead of the customary 1120° C. and results in simplification of apparatus. In this modification emergent gases are passed over copper at 900° C. to decompose and eliminate interference caused by carbon disulfide and carbonyl sulfide. Kirshenbaum and Streng (*96*) compared an isotopic method with the Unterzaucher method for determining oxygen in rubber and concluded that the latter yields low results.

Jacobs and Lewis (*80*) and Smith and Otvos (*162*) described apparatus for determining density and chemically bonded hydrogen in organic liquids from the rate of absorption of beta rays. Lake (*102*), reporting for an American Petroleum Institute committee, showed that nitrogen in petroleum and shale oil can be determined by Kjeldahl, Dumas, or ter Meulen methods by giving careful attention to indicated operating conditions. Holowchak, Wear, and Baldeschwieler (*73*) employed a nickel-magnesium catalyst in a modification of the ter Meulen method for determining nitrogen and claimed advantages over the Kjeldahl and Dumas methods when applied to petroleum feed stocks and other products containing very small amounts of combined nitrogen. Wankat and Gatsis (*179*) used high-pressure catalytic hydrogenation to determine nitrogen in petroleum distillates containing 1 to 100 p.p.m. Nickel was used as catalyst in an autoclave; the resulting ammonia was absorbed on acidic alumina and determined by the Kjeldahl procedure. Deal, Weiss, and White (*32*) described potentiometric titration methods for determining and differentiating organic bases in crudes, distillates, and residues; total nitrogen bases were determined by titration with perchloric acid in acetic acid–chlorobenzene solvent; moderately strongly basic nitrogen was determined by titration with hydrogen chloride in chlorobenzene solvent.

SULFUR AND ITS COMPOUNDS

Mottlau and Driesens (*133*) and Cranston, Matthews, and Evans (*26*) described modifications of x-ray absorption methods for determining total sulfur in hydrocarbons. Jay (*82*) indicated that a correction is necessary for interference by halogen of ethyl fluid when determining sulfur in gasoline by burning in a lamp and titrating the acidic reaction products; the correction is approximately 0.006 for each milliliter of tetraethyllead per gallon of gasoline. Elphick and Gerson (*42*) determined sulfur in coal by titrating the neutralized filtered calorimeter washings conductometrically with barium hydroxide solution.

Galloway and Foxton (*51*) detected free sulfur in rubber by the blue-green color produced in a filter paper contact procedure with sodium hydroxide and pyridine. Eccleston, Morrison, and Smith (*40*) described a polarographic method for determining elemental sulfur in crude oil. Bartlett and Skoog (*10*) determined free sulfur in hydrocarbons by dissolving the sample in acetone and causing the solution to react at room temperature with an acetone solution of sodium cyanide; the subsequent addition of ferric chloride solution produced a colored reaction product whose absorbance at 465 mμ formed the basis of the determination.

Mapstone (*121*) criticized back-titration methods for mercaptans in gasoline and recommended direct titration with standard silver nitrate solution to sodium nitroprusside as internal indicator. An amine and a solutizer should be used, respectively, to prevent precipitation of silver oxide and to increase solubility of mercaptans in the aqueous phase. Beychok (*11*) determined mercaptans in gasoline by silver nitrate titration. Prior treatment with charcoal freed the sample of interfering dye and treatment with dilute nitric acid eliminated color interference by oxidation inhibitors. Segal and Starkey (*156*) determined small amounts of methyl mercaptan in air in the presence of dimethyl disulfide and dimethyl sulfide by absorption in mercuric cyanide solution; the precipitated mercuric dithiomethoxide was weighed. Arnold, Launer, and Lien (*5*) identified naphthenic thiols in petroleum thiols by a combination of chemical separations, distillation, chromatography, and infrared spectroscopy. Hopkins and Smith (*75*) quantitatively removed thiols from petroleum distillates by reaction with sodium aminoethoxide in anhydrous ethylenediamine.

Hastings (*66*) determined aliphatic sulfides in petroleum fractions boiling below 232° C. from the ultraviolet absorption of the complexes which this class of sulfur compounds forms with iodine. He claims that 1 p.p.m. can be determined. Houff and Schuetz (*76*) described bromide-bromate titration methods for the determination of organic sulfides; Luk'Yanitsa and Nekrasov (*112*) employed potassium iodate in acetic acid for the same determination. Earle (*37*) determined disulfides in petroleum products by reducing to thiols and titrating with silver nitrate after preliminary elimination of the original thiols by reaction with acrylonitrile to form thioethers. Karchmer and Walker (*89*) studied the polarographic behavior of organic di-, tri-, and tetrasulfides and found that tetrasulfide and trisulfide produced three polarographic waves the last of which may be used to approximate concentration. The intermediate wave of the polysulfide occurred at about the same voltage as elementary sulfur and the presence of the latter in large amounts may be suspected if the polarogram of an unknown shows a ratio greater than 1 for the intermediate to late wave.

Karr (*90*) reported on a study of qualitative tests for petroleum sulfur compounds. Birch (*12*) employed aqueous mercuric acetate to separate open-chain sulfides, cyclic sulfides, and thiophenes by extraction of narrow petroleum distillation fractions. Kinney, Smith, and Ball (*95*) employed a combination of physical, chemical, and mass spectrometric methods for the isolation and identification of seventeen thiophenes in shale-oil naphtha. Levitt and Howard (*108*) determined thiophenes by oxidation with nitric acid and precipitation of equivalent barium sulfate, after preliminary removal of all other sulfur compounds except aromatic and aliphatic sulfides.

CATALYSTS

Smith (*160*) described an apparatus which simplifies the method of surface area measurement of catalysts. Key and Hoggan (*94*) described a spectrographic method for determining chromium, iron, nickel, sodium, and vanadium in silica-alumina cracking catalysts, with a rotating disk electrode composed of powdered catalyst, lithium carbonate, graphite, and cobalt as internal standard.

MISCELLANEOUS

Gouverneur and van Dijk (*58*) determined density of as little as 0.2 ml. of a liquid sample by the principle that the heights of two connected columns of different density when in equilibrium are inversely proportional to their density. Kanda and Haseda (*87*) used photoelectric measurement of light transmitted as a sensitive indicator of turbidity in the cloud point determination of lubricating oil. McCutchan and Young (*117*) described a micromethod for determining the flash point of petroleum products on as little as 0.3 ml. of sample in a flash chamber drilled in an aluminum block. They found agreement with the macro Cleveland open cup ASTM method. Patzau (*143*) described a bomb calorimetric method for thermal value of petroleum fuel, in which the sample is placed in a stainless steel or platinum cup covered with collodion. His results were 1% higher than without the collodion cover, although they were corrected for the calorific value of the collodion. This was explained by the probability that when the bomb is filled with oxygen an uncovered sample oxidizes to products of lower heat value. Fein, Wilson, and Sherman (*46*) concluded from a study of methods for predicting the net heating value of petroleum fractions that an equation based on aniline point and gravity gives results comparable to a single calorimetric determination. Scheibl (*154*) determined the molecular weight of aromatic hydrocarbons colorimetrically from their picrates. Brown (*16*) described an automatic photoelectric method for determining aniline point. Jacobs (*79*) developed an apparatus for determining automatically if a sample flashes at a predetermined temperature. Weller (*187*) determined air-fuel ratio acoustically by analysis of engine exhaust gas burned in a furnace to convert it to a mixture of two gases plus water vapor. Kusa (*101*) and White, Penther, Tait, and Brooks (*188*) described a combustion furnace for determining sulfur and halogen, in which the progress of the heating element is controlled automatically. Gibson (*54*) designed an automatic hydrocarbon-sampling system in which frequency of sampling cycles is proportional to line flow.

Weber (*183*) described a supersonic instrument for instantaneously and continuously measuring viscosity at −129° to +315° C. Honig and Singleterry (*74*) designed a moisture- and oxygen-protected viscometer useful over large viscosity range and in the study of deviations from Newtonian flow. Spear (*166*) described a falling ball-type viscometer for temperatures up to 550° C. Mill and Gates (*130*) discussed errors in results from rotational viscometers caused by increases in temperature developed at high rates of shear. Foreman (*48*) showed that low-temperature extrapolated viscosities are more accurately obtained from data from rotational viscometers than from capillary viscometers.

Black, Harvey, and Ferris (*13*) described the adaptation of an Abbe refractometer for high temperature use. Easterbrook and Hamilton (*38*) used refractometry to detect the colorless bands in adsorption chromatography and described a low cost differential instrument for making the determination.

Aldershoff, Booy, Langedijk, Philippi, and Waterman (*4*) described a nine-stage molecular distillation apparatus in which transfer from stage to stage is accomplished mechanically. Sims

(*159*) and Paschke, Kerns, and Wheeler (*140*) modified the Booy and Waterman micromolecular still, dispersing the minute sample on glass wool, although still following the distillation by changes in the extension of the quartz spiral from which the distillation pan is suspended. Trevoy and Torpey (*176*) described a high-vacuum falling film distillation unit which measures rate of evaporation to 0.5%, permits evaluation of distillate without interrupting the distillation, and is useful for detecting a fraction of a per cent of contamination in some products. Wiberley and Siegfriedt (*189*) developed a reduced volume ASTM distillation apparatus for gasoline and kerosine. Myers and Kiguchi (*135*) described apparatus for conducting ASTM-type distillations on lubricating oils and other high boiling stocks which require vacuum to prevent cracking. Feldman, Svedi, Connell, and Orchin (*47*) found that a mixture of 1-methylnaphthalene and 2-methylnaphthalene is useful for evaluating performance of distillation columns at 20 to 760 mm. of mercury and a separating efficiency of 5 to 60 theoretical plates. Preston (*149*) presented a graph for correcting the lightest component in a low-temperature fractional distillation for vapor holdup between column and receiver.

Ubbelohde, Neumann, and de Jong (*177*) presented a method for indicating the static load-carrying capacity of lubricating oil. The method is based on the thickness of the oil layer between two optically plane glass plates and the authors described an interference comparator for making this measurement. It was found that oils differed greatly in thickness of film at a given pressure.

Walker and Conway (*178*) determined hydroperoxides in petroleum fractions by refluxing with sodium arsenite solution, removing the organic layer, and titrating the excess of reducing agent in the aqueous phase with standard iodine solution. Bruschweiler, Minkoff, and Salooja (*18*) eliminated the interference of hydrogen peroxide by precipitating it in alkaline solution with lanthanum acetate before determining organic hydroperoxides by polarography. Bailey and Knox (*6*) determined aldehydes in hydrocarbon combustion products by conversion to the silver salts of the corresponding acids by passage through a column packed with silver oxide and subsequent chromatography of the liberated acids on silica gel. LeRosen, Wike, and Denton (*104*) used the dielectric constant of dewaxing solvent and solvent-oil blend to estimate the waxy oil content of the blend. Weiss, Jungnickel, and Peters (*186*) presented an outline of methods for analyzing spent sulfuric acid for many of its constituents. Hart and King (*64*) described an apparatus for detecting organic chlorides, which involved thermal cracking to produce a conducting solution which actuates an alarm system. Groennings (*59*) determined the porphyrin aggregate of petroleum by extraction with hydrogen bromide in glacial acetic acid, transfer to chloroform, and absorbance measurement at 5300 A.

Glenn and Olleman (*56*) described an involved method for determining functional groups in neutral oil from hydrogenolysis of coal, including pyrrole-type nitrogen compounds, and ether- and alcohol-type oxygen compounds. Gemant (*53*) studied the activity of exchange resins (Amberlites) on organic ions in hydrocarbons and concluded that removal of acidic and basic compounds from hydrocarbons is possible by ion exchange. Lumb and Winsor (*113*) analyzed binary mixtures—one of which may be a hydrocarbon—by phase changes that occur upon titration with a polar organic liquid in the presence of a solution of a solubilization compound. Tenney and Sturgis (*172*) studied the relative adsorbabilities of hydrocarbon classes on silica gel, alumina, active carbon, and combinations of silica gel with magnesia and alumina. Relative affinities of hydrocarbon classes for silica gel are shown and indications are given of possible separations. Smith (*161*) discussed wet oxidation of lubricating oil with perchloric and nitric acids, specified a ratio of the oxidants for best results, and reported that in one deviation from these optimum conditions a violent reaction occurred.

McArthur, Baldeschwieler, White, and Anderson (*115*) described a system for evaluating test procedures in a petroleum laboratory. This included analysis of variance, the *F* for comparing variances, Dixon's *Q* test for rejecting results, and Bartlett's test for homogeneity of variances.

ACKNOWLEDGMENT

The author gratefully acknowledges the fine work of Evelyn Grzibowski in the mechanical preparation of the manuscript and that of H. G. Sprague whose valued aid in carefully checking the text and references enhanced the accuracy of the work.

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Coatings

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THIS biennial review is intended as a selective and critical consideration of papers on the analysis of coatings, as previously defined (*19*), from November 1952 through October 25, 1954. As such, it represents a departure from the comprehensive bibliographies of the past years (*19–22, 31*). The selection was made from a large number of possible inclusions, collected from the literature over the past two years, and represents, in the reviewers' opinion, the notable highlights of this particular field. Almost all of the cited articles have been studied in their entirety, and, in some instances, proposed methods have been tested experimentally in these laboratories.

GENERAL ANALYTICAL SCHEMES FOR ORGANIC COATINGS

Shreve's discussion (*25*) of the applicability of infrared, ultraviolet, and Raman spectroscopy to the analysis of complex materials represents an excellent expression of the developing scope of modern instrumental methods.

Emphasis is placed on synthetic resins, particularly those with an end use in coatings. The origin of the various spectra are considered and the unique advantages and limitations are discussed. The Raman advantage lies in a much simpler spectrum—excellent in quantitative work—and in the applicability of the method to aqueous solutions. The infrared approach is more flexible, can be used on smaller samples, and suffers no fluorescence difficulties. The limited scope of the ultraviolet method enhances selectivity. Ultraviolet spectroscopy establishes compound class; infrared and Raman uniquely characterize the individual compound. With coating materials, all three are applied together, supplemented with chemical and other methods of analysis.

An oil-modified alkyd resin is taken as one example. The infrared spectrum of an oil-modified glycerol phthalate type shows bands contributed by phthalate esters, oil ester linkages, *o*-disubstituted aromatic ring, unesterified hydroxyl groups, and acid carbonyl groups. The spectrum types the polymer but does not yield information on specific polyalcohols nor on all modifiers present. Before ultraviolet spectroscopy can be advantageously applied, resolution into dibasic acid, oil modifier, and polyalcohol fractions by the conventional Kappelmeier procedure is a required preliminary step. Phthalic can be determined on the dipotassium salt by the Shreve (*26*) method. It has been shown (*10*) that maleic acid can also be determined on the Kappelmeier salt by polarography and, in the simpler case of unsaturated polyester resin formations, in situ. In addition, the authors prefer identification of the dibasic acids present, using the benzylamine procedure (*29*), prior to any quantitative work.

The identification of drying oil acids by ultraviolet spectroscopy is illustrated and discussed. Infrared limitations in this respect are shown by selected spectra. Infrared can be used, however, to detect styrene, rosin, phenolics, and other blending materials; recognition of such potential interference permits modification of the ultraviolet approach. It has recently been shown (*9*) how polymerized styrene can be determined directly in the presence of most oil fatty acids by an ultraviolet spectrophotometric method. Polyhydric alcohol fractions are handled by chemical methods; spectroscopic methods may be potentially useful. The spectroscopic infrared method has been applied (*24*) to the characterization of some 25 commercially available polyhydric alcohols.

Complicated, ill-defined samples require "group" analysis. Group absorption frequencies remain nearly constant with changes in molecular weight and in the remaining structure of the molecule. Hence compounds can be typed and changes in processing can be followed. Constancy of absorption holds also in ultraviolet and Raman spectroscopy. Intensity, if expressed as absorptivity per functional group, remains approximately constant in a homologous series of compounds. The principle is discussed as applied to the petroleum industry. Qualitatively, absorption changes which occur during polymerization, oxidation, or separation can be followed without exact knowledge of the various contributing chemical species.

An infrared spectrum can be considered a whole set of unique characterizing constants. Infrared spectra can serve as an empirical classification of complex materials which are poorly characterized as chemical individuals. Punched card and automatic machine sorting methods for indexing and sorting spectra have added greatly to the effectiveness of these techniques. Despite their great usefulness, however, physical and/or chemical separation procedures and various other physical and chemical methods of examination are needed in the investigation of complex coating systems. This paper has been selected as the introductory monograph because its general approach so closely parallels that of the authors of this review.

Ahlers and O'Neill (*1*) have reviewed the present state of knowledge regarding the structure of oils and resins, including some treated oils and synthetic resins. Problems still outstanding and their technical significance are indicated, and the possibility of solving them by newly developed techniques is discussed. The most important "newer" technique, absorption spectroscopy, is considered in detail. This is a very comprehensive study, wherein the emphasis is more on the problem than on the instrument. The treatment is so detailed that only a brief outline of the material covered can be included here.

After a few remarks on the techniques of absorption spectroscopy, the identification of the component fatty acids of natural oils is discussed. Ultraviolet and infrared spectroscopic methods are used in conjunction on this problem.

The spectral determination of the fatty acids illustrates the quantitative aspect of the ultraviolet approach; absorption data on pertinent acids are tabulated. In this section, great emphasis is placed on the study of cis-trans isomerism, where the infrared approach is particularly applicable.

The discussion of alkali isomerization includes the method employed, spectroscopic evaluation of the results, and the orientation limitations of this approach. Ultraviolet spectra before and after isomerization are included.

In the spectroscopic study of the formation of stand oils, the infrared method is weak; the ultraviolet technique can be advantageously used after dehydrogenation.

Oxidized oils have been widely studied by spectroscopic methods, with the most important contributions coming from the infrared approach, particularly with respect to the identification of new structures and to the examination of scission products. The section also includes a comparison of chemical and infrared methods for the determination of hydroxyl values.

The section on resins is devoted primarily to rosin, alkyds, and styrene "copolymers." Rosin and its acids are covered in great detail, and composite ultraviolet spectra are reported; types of conjugation are also considered. The potential applicability of the Raman approach is mentioned. The alkyd discussion is apparently taken from that of Shreve (*25*). The section on styrene copolymers includes some published infrared and ultraviolet contributions, and states conclusions which might be drawn. Ultraviolet and infrared spectra have been freely and effectively used as illustrations throughout this paper. The publication includes a discussion of points raised at two of the meetings before which the paper was presented.

Kagarise and Weinberger have reported on a comprehensive investigation which the present reviewers have not yet studied but which is cited here by means of the abstract from the bulletin on U. S. Government Research Reports (*12*). The infrared absorption spectra of 92 plastic and resinous materials have been studied in the region from 2 to 15 microns, and the transmittance curves are given for 57 representative materials. A useful identification procedure based on the catalog of reference papers has been devised in order to simplify and speed up the process of identifying an unknown plastic or resin. The advantages and limitations of this proposed method are discussed.

In the authors' experience, the infrared approach has been very fruitful. However, as pointed out by Harms (*8*), the physical properties of an organic material sometimes make it very difficult, if not impossible, to take advantage of infrared methods for identification. Insoluble, infusible polymers which cannot readily be mulled represent one example. Harms proposes a preliminary pyrolysis procedure for such samples. The infrared spectrum of the pyrolysis product is then compared with the spectra of known pyrolyzates. The spectrum of the pyrolyzate is reasonably discrete as a rule and frequently resembles that of the original uncured resin.

The resin sample in a borosilicate glass test tube is heated as rapidly as possible over a Bunsen flame. The liquid pyrolyzate which condenses on the sides of the tube is sandwiched between two salt plates and analyzed in the infrared instrument. The spectra of many pyrolyzates and of some of the original unpyrolyzed materials are included. Good results were obtained with, among others, an asbestos-filled molded phenolic resin, a butylated urea-formaldehyde resin, a baked epoxy wire enamel, several polyethylene glycol terephthalates, saran, Formex enamel, and tung oil wire enamel. The author claimed some success in distinguishing among different types of paper, such as flax, rice, and rag papers. Results obtained on regenerated cellulose, cellulose acetate, and cellulose acetate-butyrate, however, were somewhat inconclusive, and those obtained on melamine-formaldehyde resins were unsatisfactory. Insoluble, infusible polymeric materials represent perhaps the most difficult problem in the field of coatings analysis. The suggested approach is very promising.

Zemany (*37*) proposes a mass spectrometric approach to the analysis of the pyrolysis products of complex organic materials. If the decomposition is carried out in a reproducible manner, it can be demonstrated that the pyrolysis products are characteristic of the parent material.

Complete details, with illustrations, of the equipment required for the pyrolysis are included. To obtain simple mass spectra and to assure the complete pyrolysis of thermally stable organic materials, Zemany found it desirable, as well as advantageous, to use small samples (0.1 to 0.01 mg.) and high temperatures (about 1700° F.). In brief, the sample is heated in an evacuated pyrolysis vessel with a 6-volt television tube filament operated at 12 volts. During heating, the pyrolysis chamber is immersed in liquid air. The gaseous decomposition product, condensed by the coolant, is used as the source of the mass spectrum.

While monomer represents the major component in some instances, the pyrolysis products are sometimes very different from the parent material. For example, the pyrolyzate from polyvinyl chloride was found to include hydrochloric acid and benzene. The mass spectrum itself is characteristic of the original material, but in some cases may be very difficult to interpret. The identification of the unknown material is accomplished by comparison of its mass spectrum with the spectra of known materials, obtained under the same experimental conditions. Use of a complementary approach, such as infrared spectroscopy, would be desirable.

To demonstrate the versatility of this method in differentiating among closely related compounds, eight alkyd samples of slightly different compositions were analyzed. By comparing the position and relative intensity of the various peaks, it was possible to demonstrate differences among the samples. The reproducibility of the mass technique was tested on three samples of Formex wire enamel, varying in sample weight from 0.2 to 0.4 mg. The major peaks were similar, varying by only a few per cent in intensity.

Other applications of the technique included analyses of protein pyrolyzates for identification and composition information and following changes in Formvar during aging. The analysis of catalyst fragments, impurities, end groups, and plasticizers is most advantageously investigated by carrying out the pyrolysis at a lower temperature.

A modified form of the Zemany approach has been tried in the reviewers' laboratory with encouraging results. For those interested in the more fundamental aspects of the thermal degradation of polymers, a recent publication of the National Bureau of Standards is strongly recommended (*17*).

Krammes and Maresh (*15*) have prepared a very comprehensive procedure for the identification of textile finishes. While textile coatings may differ considerably from coatings as covered by this review, there is considerable overlap from the component point of view. Many of the identification procedures suggested in this paper might advantageously be applied to coating materials in general. The procedures presented are primarily physical and chemical tests supplemented with some microscopy. The paper is recommended for its broad coverage and for the simplicity of the quick tests which have been proposed.

Paulson (*18*) has extended the Liebermann-Storch reaction to the detection of resins other than rosin. The recommended procedure consists of adding the sample to acetic anhydride to form a 10% solution and warming slightly if necessary to solubilize. The solution is cooled, filtered, and, after the addition of 2 to 3 drops of 50% sulfuric acid, observed for initial color flash and final color.

Characteristic colorations produced by many samples of film-forming materials have been divided into five groups: violet-to-brown; brown-to-green fluorescence; neutral; red-brown or brown; and miscellaneous. Resins and compounds falling into each of the first four groups are itemized; the fifth group includes a collection of color changes and associated resins. The variety of materials to which the test has been applied indicates that an attempt has been made to include all possible coating materials.

SPECIFIC CLASSES OF HIGH POLYMERIC MATERIALS

At least five analytical papers have appeared on the subject of styrenated alkyd resins, reflecting in part the impact of these products on the field of coatings.

Swann (*35*) has investigated the possibility of determining polystyrene in styrenated alkyd and styrenated epoxy resins by selective extraction. With the alkyd type, the dipotassium salts are isolated by American Society for Testing Materials (ASTM) method of test D 563-52 and weighed, and the percentage of phthalic acid is calculated. The filtrate (comprising solvents, excess potassium hydroxide, polystyrene and its oil interaction products, drying oil soaps, and polyhydric alcohols) is evaporated to dryness in a specified manner, and the residue is dispersed in 87% methanol for 30 minutes and filtered, and the insoluble fraction dried at 110° C., then weighed as polystyrene.

Styrene-modified epoxy resins resist saponification by any medium that can be subsequently evaporated to dryness. Controlled fusion with potassium hydroxide pellets, however, permits decomposition of the epoxy, leaving polystyrene unattacked. The unaffected but contaminated polystyrene is dissolved in warm carbon tetrachloride and filtered; percentage of polystyrene is calculated from the loss in weight of the filter crucible.

Styrene monomer is not measured by either procedure, nor does it undergo polymerization under the conditions of test. Oil acids can be determined on the methanol-soluble fraction. The fusion method eliminates interference from other unsaponifiable resins, except coumarone, indene, and petroleum hydrocarbon resins. Rosins, acrylics, and phenolics do not interfere in the polystyrene determination. The accuracy of the method was checked with good results on three experimental and two commercial samples. The isolated styrenes all showed carbonyl absorption in the infrared spectra.

Since the Swann method apparently discounted the effect of polystyrene–fatty acid interaction products, with solubilities differing from those of the parent compounds, arrangement was made by the authors to submit a sample of well-interacted styrenate for examination by Swann. The results were extremely interesting. Whereas the phthalic-filtrate procedure yielded data which were low by some 20%, excellent agreement with theory was obtained when the fusion technique was employed. It was also found that excessively long saponification times in the phthalic-filtrate procedure gave higher results than the normal application. Swann concluded that it was possible that carrying out the saponification at a higher temperature may permit results approaching those of the fusion technique.

Hirt, Stafford, King, and Schmitt (*9*) have proposed a method for the direct determination of polystyrene in styrenated oils and alkyd resins. Ultraviolet spectrophotometric analysis of the ether-soluble combination of fatty acid and polystyrene, derived from a saponification-type separation, permits the determination of the polystyrene content of the fraction and hence of the original resin. The method was evaluated on a series of fatty acids which included a saturated acid, a random and a conjugated diene acid, and a random and a conjugated triene acid. Monomeric styrene present as 10, 30, and 50% by weight was polymerized in the presence of each of these acids. The results obtained on the 30% series are tabulated. For comparison, percentages of polystyrene as determined by saponification values are included, measured both as recommended by Kappelmeier (*14*) and by using the actual saponification value of the fatty acid which would of course not be possible with an unknown sample.

The ultraviolet method was found to be effective except when large amounts of conjugated triene acids, such as those from tung oil, are present. Interfering absorption due to monomeric styrene and to conjugated trienes is corrected for in the mathematical treatment, which is essentially a three-component analysis, with the data on the monomeric styrene and conjugated trienes used as corrections for the concentration of polymerized styrene. A composite plot of the absorption spectra which were used as standards in the ultraviolet spectrophotometric measurements is included in the treatment.

Kut (*16*) has recently published a three-part study of the reaction between styrene and unsaturated fatty acids. The second part was devoted to an elaboration of the method originally reported by Armitage and Kut (*2*) for separating polystyrene from styrenated and nonstyrenated drying oil fatty acids. The method was worked out on a styrenated oil prepared by refluxing in air a partially conjugated raw linseed oil with styrene in xylene in a 1 to 1 to 2 ratio for 36 hours at about 150° C., after which 94% of the styrene, as determined by solids content, was no longer present as monomer.

An extensive series of experiments led to the following separation procedure. The styrenated oil and/or fatty acids are converted to the potassium soaps, and the dry, neutral residue is isolated from the saponification mixture. To the styrenated soap is added sufficient pure, anhydrous, neutral ethyl acetate to form a 6 to 12% solution. After dissolution of the polystyrene, the mixture is heated to boiling and the soaps are dissolved by adding a saturating volume of water; a clear, bright, nonviscous solution results. Anhydrous calcium chloride is added to the still-warm solution to precipitate the fatty acids as insoluble calcium soaps. The mixture is allowed to stand overnight and centrifuged, and the precipitate is washed with ethyl acetate. The precipitation is repeated on the filtrate plus washings until no more appreciable quantities of soap are precipitated. Some 99% of the fatty acids can be separated by this method. Low acidity polystyrene is the other product. All necessary experimental details are included, together with justification of the recommended steps.

Bobalek and Bradford (*3*) point out that the above approach can be extended to styrenated alkyds, but shows some discrepancy in estimating the polystyrene–fatty acid ratio in the finished resins. The efficiency of the method was hence evaluated by radio tracer techniques, using styrene tagged with carbon-14.

The synthesis of the active styrene was accomplished by the radiocarbonation of benzylmagnesium chloride, followed by the reduction of the resulting phenylacetic acid to the alcohol with lithium aluminum hydride, and subsequent dehydration of the phenylethyl alcohol with molten potassium hydroxide. Experimental details are included. Resin synthesis was carried out by both pre- and poststyrenation, as illustrated by diagrams. The reaction conditions, details of which are included, were fixed by preliminary experiments using inactive styrene.

Experimental styrenated acids were treated with 1N alcoholic potassium hydroxide at room temperature to saponify, and the reaction product was recovered by evaporation of the solvent in an atmosphere of nitrogen. A modified Kut separation procedure was applied to this product, as illustrated by a fractionation flow sheet. Styrenated esters (triglycerides or alkyds) were given a preliminary treatment to remove phthalic acid and/or polyols. Analytical data for various runs are summarized in tabular form and discussed.

The distribution of styrene throughout the fractionation flow sheet can be established and measured consistently. There is negligible contamination of the other fractions by polystyrene and no substantial amount is lost. In some resins, polystyrene is not quantitatively separated from the fatty acids by the fractionation procedure. It may be actually bound by interpolymerization. Bobalek concluded that most of the styrene polymer in the styrenated coatings resins is virtually pure polystyrene held in solution or colloidal suspension. In addition, there was strong evidence for a reaction product of styrene and fatty acids; the quantity of this product is determined in an important way by the conditions of polymerization.

Stafford, Hirt, and Deichert (*30*) have reviewed the instrumental aspects of the analysis of styrenated alkyd resins. The treatment includes discussions of separation procedures and of instrumental methods such as those of infrared and ultraviolet spectroscopy and of polarography. As a composite example, the applicability of the instrumental procedures to the analysis of styrenated alkyd resins is illustrated on a hypothetical vehicle containing a resin made from phthalic and maleic acids, soybean oil, propylene glycol, and styrene.

Grad (*6*) has contributed an excellent paper on interpretative analyses of urea-formaldehyde resins. As the field is too broad to be presented comprehensively, the topic is illustrated by a number of typical examples.

Hydrogen ion concentration determination in both aqueous and nonaqueous media yield pH curves which are a function of type of condensation and buffer systems employed.

Important information can be obtained from viscosity with reference to aging during storage, and reactivity as measured by gel time. For solutions which are non-Newtonian, however, the results often depend on the instrument used for viscosity measurement. A discussion of instrument types and their application is included.

Very careful control is necessary in the determination of solids by the PMMA method; the most important variables are listed. Since this method involves both physical and chemical changes, the solids data obtained are empirical. For "true" nonvolatile data, the freeze-drying approach or water titration at −40° C. by the Karl Fischer method are considered promising. A plot of per cent solids *vs.* specific gravity is a straight line over a fairly large part of the curve, indicating the possible applicability of specific gravity to solids determinations.

Refractive index is another very useful physical property. Modified urea-formaldehyde resins deviate markedly from the 1.555 index of the unmodified solid resin.

The results of a free formaldehyde determination, though empirical, are significant with respect to storage life, reactivity, and behavior in final application. Sulfite titration at 0° C. gives good precision if the titration interval is short and controlled. An alternative, colorimetric method involving the formaldehyde vapors is described.

The urea content may be calculated from the Kjeldahl nitrogen but, in general, total nitrogen gives only part of the picture. Nonurea nitrogen may be present as ammonium salts and/or organic nitrogen-containing compounds. The Van Slyke test has been useful in determining primary amido groups in urea-formaldehyde resins.

Elemental analysis by fusion with metallic sodium can still be applied with advantage to the detection of nonurea formaldehyde compounds. Spectrographic and flame-photometric methods are excellent time savers in quantitative work.

Dialysis is the most revealing approach to the detection of condensation and buffer systems. A flow diagram of a dialysis extraction procedure is included.

The study also contains a section on the microscopical identification of fillers and extenders, illustrated with photomicrographs. Details on suitable selective stains are included.

For establishing the mole ratio of urea to formaldehyde in their condensation products, Grad and Dunn (*7*) have developed a procedure for the rapid and accurate determination of formaldehyde. The urea-formaldehyde resin is hydrolyzed with phosphoric acid and the liberated formaldehyde is collected in a solution containing an excess of potassium cyanide. Gravimetric and volumetric methods applicable to this solution are described in detail. A procedure to correct for the effect of interfering substances is recommended.

Urea is determined by reacting the resin with benzylamine according to the method suggested by Kappelmeier (*13*). Complete directions are given and a section on interfering substances is included.

The reviewers have found that a modified form of the described benzylamine method can be applied to urea-resin modified alkyd resins with good qualitative results. The crystalline derivative resulting from the application of benzylamine to the resin includes the *N*-benzylamides of any dibasic acids present in the alkyd and dibenzylurea. Analysis of the infrared spectrum of this mixture permits the rapid detection of both the acidic components and the urea.

Since the presence of alkyd resins in nitrocellulose lacquers makes it virtually impossible to isolate either nitrocellulose or the alkyd in pure form for gravimetric determination, Schaefer and Becker (*23*) have proposed methods for the determination of nitrogen in nitrocellulose and of nitrocellulose and combined phthalate in lacquers. The nitrate nitrogen is determined by dissolving the sample in glacial acetic acid, heating with ferrous chloride until the iron is oxidized to ferric, and titrating the ferric iron with standard titanous chloride. Assuming 12% nitrogen, the nitrocellulose content can then be calculated.

Nitrocellulose interferes with the use of the Kappelmeier procedure for the determination of combined phthalate content. Going through the reduction procedure does give a water-soluble nitrocellulose, but subsequent application of the Kappelmeier method is hindered by contaminating potassium salts. After reduction, extraction with methylene chloride is proposed; the phthalic content can then be determined by ASTM D 563-52, followed by corrected ethyl alcohol loss. If diglycollic acid is present, further modification of the drying procedure is necessary. Details are given for the method, which has been applied to a lacquer of known composition, to a commercial lacquer, to various alkyds, and to known mixtures of phthalate and diglycollate.

Swann and Esposito (*36*) have investigated the analytical problem presented by alkyd resins which have been modified with vinyl chloride-acetate copolymer. The increasing use of this modifier necessitates control methods for both major components. Because of interferences, the analyses are best performed on separated resin fractions. A study of possible selective solvents resulted in the recommended addition of dioxane or methyl isobutyl ketone to improve the solubility of the alkyd fraction before pouring the sample into 0.125N absolute alcoholic potassium hydroxide. This concentration permits precipitation of the vinyl resin without decomposition; the alkyd resin is retained dissolved. Details of the separation of alkyd and vinyl resins and estimation of the latter are given.

Chlorinated paraffin or chlorinated diphenyl stays with the filtrate. Chlorinated rubber precipitates with the vinyl and can be removed by washing with 60° C. xylene immediately after filtering and washing the separated vinyl resin.

The alkyd fraction is saponified in its solvent and the phthalic anhydride content is determined by drying the dipotassium salts at 110° C. The dipotassium salt is checked for potassium chloride, which would occur if chlorinated paraffin were present. Chlorinated diphenyl affects neither the phthalic nor the vinyl determination. If potassium chloride is present, the phthalic content can be determined by the Swann lead precipitation procedure (*34*).

If no other sources of chlorine are present, the vinyl resin content can be determined directly on the vehicle. A fusion procedure, representing a modification of Swann's styrenated alkyd approach (*35*), is recommended for chlorine determination. Although the proportions of acetate and chloride can vary considerably, the medium acetate copolymer is best suited to vinyl-alkyd blends. If medium length, the vinyl chloride content should equal 89.5 to 91.5% of the copolymer found.

Vinyl alkyds made according to specification were used as knowns to test the methods. Excellent results obtained on these samples are tabulated.

Siegel and Swanson (*27*), in discussing the x-ray diffraction patterns of the polymethylstyrenes, report results of potential analytical applicability. The physical properties of polymers of *o*-, *m*-, and *p*-methylstyrenes and of the dimethylstyrenes are similar to those of polystyrene, except for the 30° C. increase of the second order transition temperature (T_g) in the case of the *o*-methyl compound. If T_g represents the temperature at which free rotation about the C-C bond can occur, x-ray diffraction should show these differences in structure.

X-ray patterns were obtained on a number of polymers prepared from the pure isomers of monomethyl and dimethylstyrene. Except for a different intensity ratio of the two peaks, the patterns of those lacking *o*-methyl groups looked like that of styrene. When the *o*-methyl group is present, the second peak is poorly defined.

SPECIFIC CONSTITUENTS

Shay, Skilling, and Stafford (*24*) have investigated the applicability of infrared spectroscopy to the identification of polyhydric alcohols in unsaturated polyesters. The method can also be used to identify the alcohol fraction of ester-type coating resins. Three other potentially useful contributions, two on the identification of dibasic acids (*29*, *33*) and the third on the analysis of polyester resins (*32*) are also applicable, in whole or in part, to the analysis of alkyd-type coating resins.

After saponification according to ASTM D 563, the insoluble potassium salts are filtered, and the filtrate is made slightly acid with concentrated hydrochloric acid. Precipitated potassium chloride is filtered off and the filtrate is concentrated to about 5 ml., transferred to a separatory funnel with 10 to 15 ml. of water, and extracted with an equal volume of ether. The water is made slightly alkaline and dried by evaporation. The evaporation residue is extracted with alcohol and filtered, and the filtrate is evaporated. The residue represents the polyol fraction.

The infrared spectrum of the polyol fraction is compared with those of a selection of commercially available polyols. The 650- to 1550-cm.$^{-1}$ region of 24 spectra is reproduced. Composite spectra have been included to illustrate the problem presented by mixtures of some of the common glycols, and the manner in which such an occurrence can usually be solved.

It was found easy to identify a single unknown polyol. Identification of a mixture of two liquid glycols, with components present over a reasonably wide range, is almost always successful. Mixtures of a solid and a liquid glycol should be first separated by solvent fractionation. Infrared analysis is not successful in the complete identification of most mixtures of more than two polyols.

Jordan recommends (*11*) a sensitive, qualitative method for the rapid detection of vicinal diols in alkyd resins. While developing a method for the determination of water in glycols and glycerol, several solvents, including benzene, toluene, xylene, petroleum naphtha, cyclohexane, and turpentine, were found to form ternary azeotropes with ethylene glycol and water. Xylene was particularly effective in separating low molecular weight glycols from other polyols, using a Dean-Stark trap. Vicinal diols can be detected in this fraction by the standard periodate procedure.

After an ASTM D 563-type saponification and separation, the filtrate is evaporated and water added to keep the volume between 100 and 200 ml. The filtrate is acidified in a separatory funnel and extracted with ether. The combined water layers are evaporated to 150 ml., 50 ml. of xylene are added, and 100 ml. of azeotropic distillate are taken. The distillate is evaporated to 5 to 10 ml. and vicinal diols are detected by a white precipitate on addition of periodic acid followed by silver nitrate.

There is no known interference. A positive test was obtained with the vicinal diols representing as little as 5% of the polyol fraction. Since all low molecular weight vicinals produce similar effects in the properties of alkyds, no further identification is needed. This brief paper has been included here in some detail to point up the need for a method for the quantitative separation of the total polyol fraction.

Spagnolo (*28*) has modified the glycerol method based on the formation of the sodium-copper (II)-glycerol complex to increase sensitivity and extend applicability to glycerol esters and resinous vehicles. Description of apparatus and reagents and directions for the preparation of the standard calibration curve and for the analysis of aqueous and alcoholic solutions are given.

Although Beer's law is not obeyed, a linear relationship is obtained for the recommended glycerol concentrations of 17 to 65 mg., using the modified method. *Vic*-diols such as ethylene and propylene interfere by giving a blue color, whereas pentaerythritol, diethylene glycol, dipropylene glycol, trimethylene glycol, and polyethylene glycol formed no color.

Hobart (*10*) has developed a method for unsaturated acids in polyesters which is fast and flexible, and which, under suitable conditions, might be applied to the usual coating-type resins. Using polarography, Hobart determined maleic and/or fumaric acids directly on the polyester-monomer formulation as received. Phthalate ester and styrene monomer do not interfere.

An equation was derived for applying an acid number correction of the diffusion current such that per cent maleic anhydride = $I_d/0.01\ KW$, where I_d is the corrected diffusion current, K is a proportionality constant, and W is the sample weight. Complete details are given for the determination of maleate unsaturation in the presence of styrene or other monomer. (This procedure may be extended to coating-type alkyds if correction is made for interaction of the acid in any manner which would change the conjugated system originally present, such as adduct formation with drying oil acids.)

ASSOCIATED MATERIALS

Ellis and Le Tourneau (*5*) recommend a rapid, accurate absorption method for the analysis of commercial lacquers, which permits the simultaneous direct determination of aromatics, saturates, total hydrocarbons, and total oxygenated compounds. Separated fractions would probably be amenable to further analysis, if necessary. The procedure is identical with that previously described (*4*) for hydrocarbon mixtures, with the substitution of a new dye and a new displacing agent, to adapt the method to the present purpose.

Details of minor modifications of the original procedure are given. *N*-Butylamine is the new displacing agent. The new dye component, Rhodamine B base, is added to the original fluorescent indicator to mark the boundary between the oxygenated compounds and the *N*-butylamine. Two pounds per square inch gage pressure is applied to the column. After the fluorescent, orange top boundary of the sample has passed through about 25 cm. of the 3-mm. outside diameter glass tubing, the length of each section is measured.

The method was tested on four basic formula lacquer thinners and one arbitary mixture, with good results over a wide range of composition. It is also suitable for the analysis of oxygenated compound–hydrocarbon mixtures other than lacquer thinners.

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Ferrous Metallurgy

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PRACTICAL innovations in ferrous analytical chemistry during the period October 1, 1952, to October 30, 1954, might be grouped into methods more rapid than previously used and those evolved to meet specific new requirements.

X-ray fluorescence has been utilized to an increased extent. Activation and tracer techniques, though restricted for day-to-day application by lack of ready access by many laboratories to suitable sources, have become established as useful and practical. Reagents that are specific for a given metal or group of elements continue to be adopted as rapidly as they are discovered. Noteworthy in this category are cuproine and similar specific compounds for copper, mandelic acid and its derivatives for zirconium, chelating reagents, and ion exchange resins.

Spectrographic and x-ray diffraction methods have continued as two of the analyst's most versatile tools. New techniques and improved apparatus have increased their utility. The nonmetal constituents in ferrous metals have not yielded to these methods, however; such elements as carbon, phosphorus, and sulfur continue to be determined by older methods. Sufficient progress has been made during the past 2 years to warrant the hope that simpler methods will come into practical use.

Nonmetallic compounds in ferrous metals have been of active interest; principal progress recently has been in utilization of new methods developed by the analyst for research on the role of these constituents in metals.

Detailed information on recently published work in x-ray fluorescence (*3, 8, 15*), spectroscopy (*4, 13, 21, 24, 37–39, 44, 73, 83*), activation and tracer methods (*1, 6, 7, 14, 48, 49, 59, 60*), and separation methods (*5, 19, 30, 34, 56, 65, 75, 76, 84, 88*) have been published, as well as useful new reference book (*2, 50, 52, 69*).

ACTIVATION AND TRACER METHODS

Activation and tracer methods, though not extensively used in industrial work at present, have been put to practical use. Carbon, by deuteron bombardment is converted to nitrogen-13, which may be determined by the Kjeldahl method (*1*). Boron may be detected autoradiographically following neutron activation (*20*).

In the analysis of mixtures of columbium, tantalum, and titanium, activation methods have been effective (*6, 48*). For trace constituents, this method is especially useful (*49*), and as facilities for activating specimens become more generally available, it will undoubtedly find increased application. Similarly, when facilities are installed in more laboratories for accommodating the relatively low level of activity necessary, tracer methods will be more widely applied for analytical control and research.

ALUMINUM

The quantitative isolation of aluminum from other elements in ferrous metals remains the principal obstacle to its accurate determination. Complexing or sequestering agents which have been investigated include: the sodium salt of ethylenediaminetetraacetic acid (EDTA) in the presence of potassium cyanide (*14*), thioglycolic acid (*34*), ascorbic acid following a mercury cathode separation (*41*), and ammonium thiocyanate (*80, 81*). In the first three methods, aluminum is isolated from the iron complex by extraction of the aluminum hydroxyquinolate into chloroform; in the latter, the iron is extracted into an ether-tetrahydrofuran mixture. Use of polythene apparatus has eliminated the errors associated with leaching of aluminum from the glass ware.

BORON

The principal methods for determining boron in ferrous metals involve either spectrographic methods or reaction of boron with such compounds as quinalizarin, chromotropic acid, or carmine (*18, 42, 53*) in a medium of concentrated sulfuric acid or with curcumin in aqueous or organic solution (*78*). All of the above are for determining relatively low concentrations; they are subject to interference from elements commonly present in iron and steel. Isolation of the boron without excessive contamination is both time consuming and difficult. No improvement over these methods has come into use in recent years. The echelle type of grating, which recently became commercially available, provides a promising means for improving spectrographic methods for determining small quantities of boron (*44*). An autoradiographic technique using neutron irradiation also has shown promise of providing a specific method for detecting boron in steel (*20*). It is not in widespread use at present.

CARBON

Methods for the determination of carbon in ferrous metals by combustion in oxygen are well established and simple. Exceptions are procedures for the metals with high thermal stability, or with unusually low carbon contents.

New fluxes which show promise for the heat-resistant alloys include a cryolite–lead oxide mixture (20 parts of Na_3AlF_6 to 100 parts of Pb_3O_4 by weight) (*63*) and boron trioxide (2.5 parts of B_2O_3 to 1 part of sample by weight) (*61*).

The conductometric method for measuring the carbon dioxide evolved during combustion continues to gain acceptance for low-carbon materials (*85*). For highest precision with very small amounts of carbon, the carbon dioxide is trapped in liquid nitrogen, the oxygen is removed, and the amount of carbon dioxide remaining is measured by means of the pressure change of a sensitive manometer (*67*).

CERIUM

A method for isolating the cerium group (rare earth) elements and estimating the amounts of each involves removal of iron and major steel components with the mercury cathode and concentration of the rare earths by precipitation with ammonium hydroxide using an iron carrier. The combined hydroxides are dissolved in hydrochloric acid; uranium and sodium chlorides are added as an internal standard and spectroscopic buffer, respectively. The specimens are then excited with a direct current arc and their spectra recorded. The procedure is applicable to 1-gram samples of steel containing as little as 50 mg. of rare earths (*82*).

In another procedure (*83*) the cerium is coprecipitated with barium as the fluoride in a hydrochloric-perchloric acid solution. Quantities as small as 0.003% of cerium in stainless steel may be determined.

COLUMBIUM

Methods for separating columbium from tantalum and for determining each have continued to receive attention. Diisopropyl ether (*19, 30*), diisopropyl ketone (*84*), ethyl methyl ketone (*36, 56*), and mixed butyl phosphoric acids in di-*n*-butyl ether (*76*) were found to be specific for extracting these elements from acid solution and a basis for their separation. The value of activation and tracer techniques using columbium-95 and tantalum-182 for evaluating the separation procedures was established (*7, 59, 60*). The columbium thiocyanate (*54, 88*), columbium-pyrogallol (*35*), and columbium-hydroquinone (*40*) complexes were explored and practical methods for their utilization in determining columbium described.

COPPER

The reactions of copper with diethylammonium diethyldithiocarbamate and the more specific reaction with cuproine and neo-cuproine (*16, 22, 28, 33*) have provided improved methods for determining small amounts of copper in ferrous metal. The former requires use of ethylenediaminetetraacetic acid as a masking agent; the latter utilizes the citrate or tartrate to complex the iron prior to extraction of the copper-organic compound from aqueous solution. Of the extractants tried, chloroform seems most effective. Ethyl alcohol is added to the extract to promote color formation.

GASES AND NONMETALLIC COMPOUNDS

A micro-vacuum fusion unit for determining gases in metals with an accuracy of ±10 p.p.m., on 50- to 200-mg. specimens has been utilized (*27*). The problem of determining gases in high-sulfur materials by the vacuum fusion method has been recognized (*29*). Apparatus for research on gas analysis methods was devised (*32, 77, 89, 90*).

A method for determining oxygen in metals based on irradiation of the oxygen to produce radioactive fluorine-18 has been studied (*66*).

Nonmetallic compounds were studied by isolation methods (*25, 46, 55, 57, 86*) and spectrographically (*38, 39*). No fundamentally new methods were described.

PHOSPHORUS

By the use of the direct-reading spectrograph (*13*), phosphorus contents as low as 0.005% were determined successfully.

For high purity iron (*23*) the molybdenum blue method was utilized after removal of iron with the mercury cathode. After reduction to the ferrous state, a cation exchange resin (*91*) was used to separate iron from phosphorus. The efficiency of the separation was checked by use of phosphorus-32 as a tracer.

SLAGS AND REFRACTORIES

The combustion method, when details of the empirical procedure are established and followed closely, provides a means for rapid determination of sulfur in blast furnace slags (*10*). Dehydration with perchloric acid following fusion with sodium carbonate (the fusion requires heating 10 minutes at 450° and 15 minutes at 1000° in a electric muffle furnace) provides a rapid method for determining silica in blast furnace slags (*12*).

By use of semimicromethods (*17*) or spectrographic procedures (*71, 73*) a complete analysis of silicates and slags may be effected quickly; only 100 to 500 mg. of sample are necessary. Calcium and magnesium in limestone were determined by titration with ethylenediaminetetraacetic acid (*43*).

The use of pH measurement for evaluation of the basicity of slags has received renewed attention (*74*).

SULFUR

Emphasis on development of a rapid stoichiometric method for the determination of sulfur has continued. Evaluations of combustion procedures continue to indicate that, though reproducible, they must be standardized against materials of similar composition and with a range of sulfur contents comparable to those of the unknown samples (*11, 51, 70, 72, 79*). Recovery of the sulfur content is reported to range from 86 to 100% of the sulfur present. Temperatures required for combustion range from 1300° to 1425° C. and oxygen flows from 1 to 3 liters per minute.

Work has been done to simplify and improve the gravimetric (*9*) and the evolution methods (*31*). Phosphoric has been used in place of hydrochloric acid to liberate the sulfur as hydrogen sulfide from chrome, chrome-nickel, and the nickel steels. In the gravimetric method, iron has been removed prior to precipitation of barium sulfate by passing a perchloric acid solution of the steel through an alumina column specially treated with ammonia (*64*). Iron is retained in the column and the sulfur is precipitated in the eluate as barium sulfate.

TITANIUM

Following removal of iron with the mercury cathode, titanium was determined polarographically using a supporting electrolyte 1.0, 0.5, and 1.2M in tartaric acid, sulfuric acid, and ammonium sulfate, respectively (*26*). By use of ethylenediaminetetraacetic acid, titanium was separated from interfering elements and determined by measuring the absorption of the peroxide complex (*68*). Chromotropic acid was utilized also (*47*).

ZIRCONIUM

Mandelic acid and its derivatives continue to be studied as precipitants for zirconium (*49, 58*). Generally, the precipitate is ignited to zirconium oxide and weighed. It may be dissolved in aqueous ammonia and the zirconium determined volumetrically (*87*). Benzilic (*45*) and tetrachlorophthalic acid (*62*) also may be used as precipitants in the presence of iron and elements commonly present in steel.

SUMMARY

The literature cited in this review includes only a limited number of the articles published in the period from November 1, 1952, to October 30, 1954. It is intended to include methods with specific application, which appear to be finding increased practical use. Mention has not been made of methods for analysis of the newer metals and alloys, many of which are now produced commercially and used in a manner somewhat analogous to ferrous metals.

It will be appreciated if important omissions are brought to the attention of the editors, so that they may be included in a future article.

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Nonferrous Metallurgy

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PUBLICATION of research on nonferrous metallurgical analysis has continued at a uniform level of activity for the past 5 years including the interim since the previous review (*131*) and up to August 1954. Emphasis on newer and more effective methods of analysis is on the increase, and there is reason to expect this apparent trend to be even more noticeable as consolidation of recent developments proceeds during the next few years. There has been a steady rise in the proportion of work done in foreign laboratories, now corresponding to about 60% of the total publications. Extensive reviews on the analytical chemistry of titanium (*31*), beryllium (*187*), and uranium (*153*) reflect a pronounced increase in the volume of work on the chemical analysis of these materials.

Recent advances in x-ray fluorescence analysis are rapidly bringing this method into prominence. Because of the relative independence of x-ray excitation on metallurgical structure and the ease of analyzing comparatively large surface areas, the method is not subject to the rigid requirements for sample preparation characteristic of spectroscopic methods. X-ray fluorescence analysis is, in general, less sensitive than emission spectroscopy. In an air medium, the most favorable situation is the determination of high concentrations of elements of atomic number greater than 22. Recent developments in production of crystals and in the use of a helium path promise to extend the method to lighter elements and to lower limits of detection.

One of the most interesting developments in spectrographic analysis of metals is the excitation of molten samples. Analysis of certain metals has been complicated heretofore by variables associated with structure, including grain size, distribution of constituents, porosity, microscopic shrinkage cracks, and other effects produced during solidification, heat treating, and working. A new technique involving excitation of molten metal was proposed by Frederickson and Churchill which erases these so-called metallurgical effects and eliminates undesirable differences in spectral excitation characteristics resulting from variations in previous treatment of the metal (*51*). Although developed primarily for analysis of aluminum alloys, this general technique should be valuable in the analysis of other metals in which metallurgical history of the sample influences excitation.

A double-focusing mass spectrograph for determining impurities in solids was described by Hannay (*70*). Samples in the form of wires or rods are excited by a spark discharge, giving a wide spread in initial energies requiring both magnetic and electrostatic focusing. Using photographic plates, this instrument can determine 0.1 p.p.m. of antimony and other elements in germanium with exposures of 3 to 10 minutes. For higher concentrations, electrical ion detection is used.

Polarographic and colorimetric methods have been widely used for determining impurities in all metals and in the semiconducting nonmetals. Measurement of gas in metals by vacuum fusion methods has also received considerable attention. Several new and promising applications of neutron activation were reported.

Bricker, Furman, and McDuffie developed a general technique for the separation of trace amounts of various metals using a small mercury cathode (*18*). After electrolysis, the mercury amalgam is subjected to distillation, after which the metallic residue is analyzed colorimetrically to determine nonvolatile constituents. Among the elements which can be satisfactorily removed from mercury cathodes are cadmium, cobalt, copper, iron, nickel, and zinc. The method has been applied to uranium, calcium, magnesium, and other light alloys.

ALUMINUM

An x-ray diffraction method suitable for large scale bauxite exploration studies was described by Black (*13*). Diffracted intensities are converted to mineral percentages using calibration curves based on samples analyzed chemically. Gibbsite, boehmite, kaolinite, quartz, and total iron minerals are determined. Total water, silica, iron oxide, and alumina are then computed from corrected mineral values. Computation, handling, and listing of data are facilitated by a printing-type recorder, calculating board, and automatic computer. Among the advantages of the x-ray method in exploration work are a fivefold reduction in time compared to chemical methods, reduced over-all cost by elimination of many chemical determinations otherwise necessary, identification of the minerals present leading to advance information concerning performance of bauxite in the Bayer process, and use of very small samples.

Characterization of hydrated aluminas by infrared spectroscopy and application to the study of bauxite ores were reported by Frederickson (*50*). Alpha monohydrate (boehmite), alpha trihydrate (gibbsite), beta monohydrate (diaspore), and beta trihydrate (bayerite) were shown to possess hydroxyl groups with sufficiently different absorption patterns in the OH stretching and deformation regions to serve as a basis for their identification. Infrared spectra of pure hydrates were used to identify gibbsite and boehmite in a variety of bauxite ores and diaspore in certain clays using mineral oil mulls and pressed potassium bromide plates.

Interest in the nonmetallic impurities in aluminum metal continued at a high level. Griffith and Mallett developed a modification of the tin fusion method for determining hydrogen as low as 0.1 ml. per 100 grams in wrought aluminum welds (*65*). This work is of particular interest because multiple determinations permit the determination of not only the amount but also the distribution of the hydrogen.

Kostron reviewed procedures for determining gas content of aluminum alloys, pointing out the necessity of developing a correlation between precise vacuum fusion and rapid test methods (*95*). Methods for determining oxygen in aluminum and other metals were also reviewed by Fischer and Bechtel, including hydrogen reduction, bromine in methanol, cupric chloride, hydrogen chloride volatilization, and metallographic methods (*48*).

A combustion method for determining carbon was described by Goto and Takeyama (*61*). This is a combination method providing for combustion of methane produced by hydrolysis of carbide and separate combustion of any insoluble carbon left after dissolving the sample in ferric chloride.

A method for determining boron in aluminum-boron alloys without distillation was described by Eipeltauer and Jangg (*43*). Aluminum is precipitated twice with sodium hydroxide, after which the mannitol method is used in titrating the borate. A method for determining silicon in aluminum by measurement of electrical conductivity was reported (*138*). In the range 0.1 to 0.2%, agreement with chemical determinations was within ±2%.

Conventional colorimetric procedures for copper, iron, silicon, manganese, titanium, and bismuth were adapted to analysis of aluminum alloys by Bednara (*7*). Jean studied other published methods for determining copper, iron, silicon, manganese, magnesium, zinc, nickel, chromium, tin, bismuth, zirconium, and cobalt (*85*, *87*). Sodium in aluminum and other metals was determined by Shigematsu and Kimura following precipitation of aluminum with dry hydrogen chloride and separation of sodium with uranyl nickel acetate (*163*, *164*).

Jean applied the methyl violet method for antimony to aluminum, zinc, and tin alloys down to 0.015% antimony (*86*). Colorimetric methods for iron in aluminum were studied by Mázor and Grusz using bipyridine, phenanthroline, thiosalicylic acid, dimethylglyoxime, Ferron, and thioglycolic acid (*113*). Bipyridine and phenanthroline were recommended. An interesting method for the simultaneous determination of aluminum and magnesium was described by Boettcher and Hellwig (*15*). Using Eriochromecyanine and Titan yellow, a crossed grid of isochromatic lines was plotted in terms of concentrations of the two elements. This method was found useful with binary alloys containing comparable amounts of both constituents. A rapid method using nitroso R salt was proposed by Stross and Stross covering the range 0.1 to 2% cobalt (*176*).

Methods for the polarographic determination of zinc in aluminum were adopted by British Standards Institute for the range 0.02 to 3% zinc (*19*). The sample is dissolved by successive treatment with sodium hydroxide and sulfuric acid containing potassium perchlorate. The two solutions are mixed prior to obtaining the polarographic curve.

Spalenka determined small amounts of zinc in aluminum and magnesium alloys using potassium thiocyanate as the supporting electrolyte (*172*). With a standard prepared from a solution of the sample containing a measured amount of added zinc, the method is sensitive to 0.01%.

Mills and Hermon determined nickel in aluminum polarographically after separating the nickel by precipitating with sodium hydroxide and converting it to the perchlorate (*119*). A pyridine–pyridinium chloride supporting electrolyte was used. Chromium was determined polarographically, using an electrolytic separation to remove copper if present in amounts greater than 0.25% (*118*).

Stross developed a polarographic method for cadmium in the range 0.01 to 5%, applicable to samples containing several per cent of manganese, magnesium, zinc, nickel, chromium, titanium, tin, calcium, bismuth, zirconium, antimony, silver, and niobium (*175*).

Graham and Hitchen developed a precise and accurate method for the polarographic determination of titanium in a variety of aluminum-base alloys using as the supporting electrolyte tartaric acid, sulfuric acid, and ammonium sulfate (*62*). The sample is dissolved in sodium hydroxide, followed by extraction of the titanium from the residue with sulfuric acid. A rapid method for determining bismuth, copper, and lead in aluminum alloys was reported by Norwitz, based on selective electrodeposition to remove copper from a nitric acid solution containing hydrogen peroxide (*133*). Copper and bismuth are deposited from nitric acid solution in phosphoric acid. Copper, bismuth, and lead are deposited from a hydrochloric acid solution containing hydroxylamine. Bismuth and lead are then calculated by difference.

An isotope dilution method using zinc-65 as the radioactive tracer was used by Theurer and Sweet on samples containing from 0.02 to 1.5% zinc (*178*).

Carrara described a procedure for the determination of gallium based on a preliminary ether extraction followed by electrolysis using platinum gauze cathodes in an alkaline solution containing ammonium sulfate (*25*). The deposit of gallium is weighed after oven drying. Gastinger determined gallium in aluminum alloys using ether and cupferron separations followed by fusion with sodium hydroxide to remove iron (*54*). Final measurement of gallium was made using 8-quinolinol.

A rapid titrimetric method for magnesium using sodium ethylenediamine tetraacetate was developed by Sergeant using Eriochrome Black indicator (*162*). Samples are dissolved in sodium hydroxide to remove the bulk of the aluminum, silicon, and zinc, followed by an ammonia precipitation in the presence of bromine. Cyanide is added to complex any residual copper or nickel remaining after the previous separation. Faller used the same titrant and indicator for the rapid determination of zinc at pH 9 (*45*).

A titrimetric method for magnesium was developed by Mannchen involving formation of sodium magnesium fluoride followed by potentiometric titration of excess sodium fluoride with standard ferric chloride at pH 4 (*106*). Aluminum is first removed by dissolving the sample in sodium hydroxide. Vacuum equipment for the distillation method of determining magnesium (and zinc) by the method of Urech and Sulzberger was improved and is available commercially (*186*). This equipment permits the determination of magnesium in 19 samples simultaneously.

Methods for the determination of 0.06 to 0.08% zirconium in aluminum alloys using *p*-halomandelic acids were described by Papucci, Fleishman, and Klingenberg (*141*). Excellent agreement with the phosphate method was obtained and no apparent advantages were observed between the chloro and bromo acids.

For the determination of small quantities of vanadium in aluminum, preliminary concentration with cupferron was proposed, followed by oxidation with permanganate and back-titration with ferrous sulfate using diphenylamine (*38*). Clark and Stross developed a semiquantitative spot test for tin based on decolorization of starch-iodine reagent and suitable for sorting scrap (*29*).

MAGNESIUM

Atchison and Beamer applied neutron activation analysis to the determination of trace elements in high-purity magnesium metal (*4*). Sensitivities in the order of 10^{-9} gram of phosphorus, 10^{-8} gram of copper and iron, 10^{-7} gram of sodium and potassium, 10^{-6} gram of strontium, calcium, and chromium, and 10^{-5} gram of sulfur were reported for 4-week irradiation periods. Chemical procedures were developed for separation of these elements from irradiated magnesium.

Volpi described a method for the polarographic determination of antimony, lead, zinc, and manganese in magnesium alloys (*188*). An indirect polarographic method for zirconium based on precipitation as the *m*-nitrobenzoate followed by measurement of the wave height corresponding to the first reduction of the nitrobenzoic acid was developed by Graham, Van Dalen, and Upton (*64*).

A rapid colorimetric method for zirconium in magnesium alloys using alizarin red S was reported by Wengert for the range 0.1 to 1%, with accuracy corresponding to that obtainable gravimetrically (*190*). Mayer and Bradshaw used the same reagent for this determination, showing it to be highly useful for minerals and ferrous alloys as well as magnesium (*111*). They also employed this method for determining the approximate composition of hafnium-zirconium mixtures.

Wengert, Walker, Loucks, and Stenger developed procedures for determining thorium and rare earth elements in magnesium, using a preliminary separation with benzoic acid and ammonium sebacate followed by precipitation of the oxalate (*191*).

Strange determined lithium in magnesium by flame photometry in the range 1 to 10% with errors of only 0.25 to 2% of the amount present (*174*). In order to compensate for the suppressing effect of magnesium and aluminum, addition of these elements to the standard solutions was recommended. Robinson and Ovenston used a double-beam flame photometer for determining lithium in the range 11 to 14% (*152*). Their method employs potassium as internal standard, and this technique is recommended for general use in the determination of alkali metals. Errors were less than 1%. Sodium and potassium were found not to interfere, and this was true also of silver, zinc, aluminum, and cadmium, provided the total did not exceed more than 10%.

Milner and Woodhead studied the titrimetric determination of aluminum using ethylenediaminetetraacetic acid, titrating the excess with a standard iron solution, using salicylic acid indicator (*128*). Up to 60 mg. of aluminum can be determined by this method with an error of less than 1%. A preliminary separation of aluminum as the benzoate is required in the analysis of magnesium, copper, and zinc alloys. Mayer, Bradshaw, and Deutschman described a rapid and promising procedure for determining zinc based on titration with ferrocyanide using an electrometric setup employing polarized platinum electrodes (*112*). Typical results agreed to within 0.03% at a level of 4.8% zinc.

COPPER

Considerable work was done on the application of colorimetric methods to the determination of minor constituents in copper-base alloys. Silverstone and Showell studied the determination of iron, nickel, and manganese employing a single sample (*170*). Copper is removed by precipitation with aluminum along with any lead present. Iron is determined with thiocyanate, nickel with dimethylglyoxime, and manganese with potassium periodate.

Wiedmann and Nufer determined chromium in copper-chromium alloys using the persulfate method following electrodeposition of copper (*194*). Low concentrations of copper were determined colorimetrically and larger amounts titrated. Molybdate methods for determining phosphorus, arsenic, and silicon in copper and in nickel, following selective extraction with various mixtures of chloroform and butanol, were reported (*47*). For arsenic and for small amounts of phosphorus and silicon, reduction of molybdenum with stannous chloride was recommended. Further work on the molybdate method for silicon in copper alloys covered the range up to 5% in the presence of 0.05% phosphorus and 1% iron and arsenic (*1*).

Additional work on the aluminon method for beryllium in copper-beryllium alloys was reported (*59*). Beryllium was first separated from a tartrate solution with guanidine carbonate. A permanent color standard containing ferrous ammonium sulfate and methyl red was used for this procedure.

Pohl determined aluminum in copper and its alloys, first separating interfering elements with sodium hydroxide and measuring the aluminum with Eriochromecyanine-R (*145*). Minimum deviation from gravimetric results was 0.03% in the range of 0.01 to 10% aluminum. Other photometric methods for arsenic and copper using phosphate (*41*) and sulfite with stannous chloride (*16*) were also reported.

Lutwak proposed some modifications of the ASTM method E 62-50T for determining phosphorus based on measurement of the phosphovanadomolybdate color (*103*). If the color is allowed to develop at room temperature, stability is improved and fading eliminated. Separation of metastannic acid in the final solution is prevented by using a suitable mixture of nitric and hydrochloric acids. The same procedure is recommended for both large and small amounts of phosphorus.

Goto and Kakita studied the determination of antimony in the range 0.0002 to 0.04% based on reaction with methyl violet and extraction with amyl acetate (*58*). A prior separation by coprecipitation with manganese dioxide was used on copper, lead, zinc, cadmium, bismuth, nickel, and iron.

Existing methods for the determination of tin in bronzes were reviewed by Besson and Budenz (*10*). They recommend precipitation of metastannic acid in hot nitric or perchloric acid followed by volatilization of the iodide. The ammonium iodide is specially prepared in order to exclude nonvolatile constituents. The authors were unable to confirm other work indicating that silicon tetraiodide is volatilized along with the tin. Antimony, being volatilized with the tin, must be determined separately. The method is not suitable for phosphor bronzes (*9*).

A potentiometric method of titrating tin, copper, and antimony in bearing metal and bronze involves reduction of these metals with chromous chloride, after which all three elements are titrated in one solution and the end points read from the titration curve (*27*). Rapid titrimetric methods for determining tin in various copper-base alloys based on reduction with hypophosphorous acid (*88*) and orthophosphorous acid (*146*) were reported.

Several methods for determining aluminum in brasses and bronzes were described, all based on existing methods modified for particular materials. Gwatkin used a mercury cathode separation, after which aluminum was precipitated with 8-quinolinol and titrated with potassium bromate (*68*). Wiedmann used the same reagent in conjunction with a sulfide separation, determining the aluminum gravimetrically (*193*). Miller, Gedda, and Malissa determined aluminum in copper alloys with 8-quinolinol, using sodium diethyldithiocarbamate to separate interfering metals (*116*). This was done by precipitating the undesired constituents and filtering or by extracting the complexes with a solvent. The extraction method was preferred. Kinnunen and Merikanto determined aluminum by a fluoride titration carried out in 50% Cellosolve using ferric salicylate indicator (*89*). This method gives good results in the range 0.3 to 11%.

Selective reduction of copper, iron, and titanium using zinc, lead, and bismuth amalgams was used as a basis for determining these elements (*198*). For the determination of nitrogen in copper and titanium alloys, Codell and Verderame proposed digestion with perchloric acid followed by distillation and titration with sodium hydroxide (*33*). This method is satisfactory for determining nitrogen as low as 0.01%.

A critical study of the electrodeposition of copper and zinc in brasses was reported by Tschanun, in which he investigated various types of plated and unplated electrodes, time, temperature, and nature of the electrolyte (*184*). Procedures suitable for microanalysis were recommended. Polarographic methods for determining zinc (*84*, *196*) and copper were reported. Zábranský described a polarographic method for determining bismuth after extraction of copper from a cyanide solution with dithizone (*199*).

A flame photometric method for determining indium in aluminum-bronze alloys was described by Meloche, Ramsay, Mack, and Philip using the indium line at 451.1 mμ (*114*). Indium was determined to within ±0.1% in the range 0.2 to 10%. Addition of copper and aluminum to the standards was recommended. With an oxyhydrogen burner, the minimum detectable concentration of indium in solution is about 0.2 p.p.m.

ZINC

Sampling of various zinc- and lead-containing materials was reviewed by Ensslin, and procedures applicable to melts, ingots, scrap metals, slags, and drosses were recommended (*44*). Melting, dip sampling, and granulation were found to be the most successful means of avoiding segregation.

Refinements in the dichromate methods for determining metallic zinc and zinc dust were described by Imai and Suehiro, who called attention to the importance of adding acid at intervals of 10 minutes in order to achieve complete reaction with the free metal (*82*, *83*). A sulfide photometric method for thallium in flue dust was studied by Handley and applied also to zinc assay solutions following titration of zinc with ferrocyanide (*69*).

Pellowe and Hardy applied the aluminon method to zinc metal for the determination of 0.001 to 0.01% aluminum (*142*). Thioglycolic acid was used to suppress interference by iron, copper, and tin. Interference by other metals was discussed along with modifications for using this procedure on certain tin and cadmium alloys.

Margerum and Banks studied the determination of iron with 1,10-phenanthroline from the standpoint of interference by vanadium, chromium, manganese, nitrogen, and zinc (*109*). By taking advantage of the differences in stability of the phenanthroline complexes of these elements together with their different rates of formation, it is possible to extract the ferrous complex with nitrobenzene and avoid interference otherwise encountered. With zinc it is not necessary to perform the extraction if zinc is converted to an ammine complex, and the pH is adjusted to 9. The proposed methods are not applicable to cobalt and copper because of the high reaction rates of these elements with phenanthroline and high stability of the complexes.

A rapid method for determining up to 20% uranium in zinc was described by Fritz and coworkers (*53*). No separations are required, uranium being reduced with a lead reductor followed by titration with ceric sulfate. Zinc is titrated with ethylenediaminetetraacetic acid using ammonium purpurate indicator. Interference by uranium is avoided by forming a carbonate complex.

Semerano and Mendini investigated the polarographic determination of trace elements in pure zinc, including bismuth, copper, antimony, lead, tin, indium, and cadmium (*161*). Sensitivity of the polarographic method was reported to be higher than the spectrographic method in all cases except bismuth and copper.

Reinacker and Hoschek studied the polarographic determination of indium in zinc materials at concentrations of about 0.005% (*149*). Preliminary separation of indium by ammonia precipitation in the presence of bismuth or iron is recommended, particularly if cadmium is present. Maraghini also studied the determination of indium in zinc residues using an ammonia or sulfide separation to remove interfering constituents (*108*).

A critical study of the determination of zinc in zinc-cadmium alloys by spectrographic, electrometric, and polarographic methods was published by Scheller and Treadwell (*159*). A sulfide separation of cadmium is necessary with the chemical methods.

Sietnieks determined tin in zinc die-casting alloys by the polarographic method following separation of metastannic acid and fusion of the residue with sodium hydroxide (*165*). A method for determining 0.0005% oxygen in metallic zinc was based on reaction of the oxide with hydrogen sulfide and adsorption of the water evolved in phosphorus pentoxide (*72*). The reaction takes place at 550° C.

LEAD

Although much of the recent work on an analysis of lead is concerned with colorimetric and polarographic methods, several titrimetric procedures have been reported. Mannelli proposed a titrimetric method for silver in lead using standard potassium iodide in the presence of ammonium acetate with bromophenol blue adsorption indicator (*107*).

Klenert studied the iodometric method for determining tin in antimony-lead-tin alloys containing up to 20% tin (*91*). Platinum or chloroplatinic acid was used to control dissolution of the sample. For alloys containing up to 18% antimony, bromate titration at 95° C. was recommended by Cimerman and Ariel (*28*).

Schulten described a method for determining total lead in lead dross and scrap based on treatment of the sample with sulfuric acid and boiling the residue with ammonium carbonate, followed by dissolving with nitric acid and precipitation as the sulfate (*160*).

Various possible methods for the analysis of bismuth-lead eutectic alloys containing 55% bismuth were reviewed by Silverman and Shideler, and a procedure was recommended involving preliminary separation of lead as the chloride, followed by a cupferron separation and final measurement as the phosphate (*168*).

Fraser, Beamish, and McBryde investigated the determination of small amounts of palladium in lead assay buttons and developed a satisfactory procedure avoiding separation of lead as sulfate (*49*). Palladium was separated successfully by a double precipitation with salicylaldoxime and by extraction with chloroform as the dimethylglyoxime complex. Final measurement was made colorimetrically by the iodide method.

Luke applied the rhodamine B method for antimony to samples of lead containing 0.00005 to 0.005% (*101*). This is an excellent method because of the high specificity for antimony, provided the procedure is properly carried out. Ceric sulfate is added to oxidize antimony, and it is essential that the rhodamine B be added immediately in order to avoid conversion of antimony to an apparently nonreactive state.

Luke also determined aluminum in lead, antimony, and tin alloys colorimetrically using aluminon in conjunction with a preliminary mercury cathode electrolysis, cupferron extraction, and final separation of aluminum by extraction with 8-quinolinol in chloroform (*100*).

Pohl determined bismuth in lead and its alloys colorimetrically with thiourea following a preliminary separation of lead as the bromide (*144*). Sodium fluoride was used to mask the effect of antimony, resulting in a considerable saving of time over the method involving volatilization of antimony chloride.

Bode and Henrich described a method for determining small amounts of bismuth in lead by extraction of the cupferrate with chloroform and colorimetric measurement as the iodide (*14*).

Stolyarova proposed the determination of 0.01% bismuth in lead by ultraviolet absorption of the bromide at 365 mμ (*173*). This method offers interesting possibilities, inasmuch as interference by other metals likely to be present is not serious.

Miller, Acampora, and Norwitz determined copper in lead with rubeanic acid, which has advantages over other reagents for determining copper, particularly in samples containing iron (*117*). Brown discussed the colorimetric determination of tellurium and suggested improvements in the existing methods (*22*). Preliminary separation of lead is unnecessary.

Hourigan and Robinson determined antimony polarographically and studied the effects of varying concentrations of lead, hydrochloric acid, and alkali salts (*79*). Best results were obtained in a potassium chloride supporting electrolyte. Yonezaki developed rapid polarographic methods for determining copper, antimony, lead, cadmium, nickel, zinc, cobalt, and iron in Babbitt metal (*197*). Polarographic methods for antimony, bismuth, copper, iron, and zinc in pig lead were also reported (*195*). Bishop and Liebmann determined aluminum and zinc polarographically in tin-lead alloys following preliminary separation of the lead as chloride and further treatment by chromatography on a cellulose column (*12*).

Qualitative tests for tellurium (*21*) and bismuth (*24*) in the presence of lead were reported.

TIN

Farnsworth and Pekola reviewed the present status of tin analysis covering the determination of copper, aluminum, lead, silver, arsenic, antimony, and iron (*46*). Sampling of pig metal by granulation was described in detail. Using this method of sampling, segregation of most impurities is avoided.

Coppins and Price reviewed various methods for the colorimetric determination of impurities in tin and recommended methods for lead, copper, bismuth, iron, nickel, cobalt, aluminum, zinc, cadmium, and sulfur (*34*). Ota recommended thiosalicylic acid for the colorimetric determination of iron in Babbitt metal in conjunction with separation of antimony and tin by distillation of the bromides (*140*). Suzuki determined tin and antimony in their mixtures using ceric sulfate in a dual titration procedure, calculating antimony by difference (*177*).

Silverman and Gossen found the amalgam method for oxygen in tin applicable at concentrations of 0.0001% and less, in micro samples of 2 to 5 mg. (*166*). After separation of the oxide with metallic mercury under vacuum, tin is determined in the oxide colorimetrically using an improved phosphomolybdate method.

NICKEL AND COBALT

Graham, Hitchen, and Maxwell determined titanium polarographically in nickel-base alloys and steels, using preliminary electrolysis with a mercury cathode to remove copper and iron (*63*). Good precision was achieved on samples containing 0.2 to 2.4% titanium in the presence of iron, nickel, chromium, copper, manganese, and aluminum.

A gravimetric method for determining niobium in alloys of nickel, cobalt, and chromium was reported by Golubtsova (*57*). Niobium is hydrolyzed in 2% hydrochloric acid and can be determined in the presence of 6% titanium or 1.5% vanadium. For large amounts of vanadium the niobium pentoxide is fused and reprecipitated. Tungsten in complex alloys of nickel, cobalt, and chromium was determined gravimetrically using 2,5-diamino-7-ethoxyacridine (*56*).

Bucklow and Hoar studied the polarographic determination of tungsten and cobalt using an "internal standard" method in which a measured amount of the element being determined is added to a solution of the sample for calibration purposes (*23*).

TITANIUM AND ZIRCONIUM

In a series of seven papers, Codell and coworkers summarized the present status of titanium analysis and proposed procedures for determining silicon, calcium, aluminum, molybdenum, phosphorus, and boron (*31*). Silicon is determined as molybdenum blue following separation of titanium based on a hydrolytic precipitation from fluoboric acid solution. The method is applicable in the range 0.005 to 1.5%. Calcium from 0.005 to 0.3% is determined gravimetrically or nephelometrically after precipitating the sulfate in methanol. A preliminary separation of titanium by extraction as the cupferrate is required. Aluminum is determined in the range 0.01 to 0.4% following a cupferron precipitation. Conditions were established for precipitation of molybdenum sulfide for determining 0.25 to 10% molybdenum. A polarographic method covers the range 0.0003 to 5%. Phosphorus is determined in concentrations 0.005 to 0.5% by the molybdenum blue method, and boron from 0.0004 to 0.1% is determined colorimetrically with dianthramide after distillation.

Thompson discussed the determination of iron, magnesium, manganese, nitrogen, and chloride in titanium, and proposed modifications for determining iron and nitrogen in cast and wrought forms (*180*). Thompson also described a direct method for assay of titanium metal based on titration with ferric alum following reduction of titanium with amalgamated zinc (*179*). Excellent results between laboratories were reported on titanium from 99.4 to 99.8%.

Goto and Takeyama described methods for determining total titanium, oxide, carbide, nitride, silicon, iron, aluminum, manganese, and magnesium (*60*). Corbett also discussed methods for these determinations in addition to fluoride, tungsten, molybdenum, copper, cobalt, chromium, tin, tantalum, and silver (*35, 36*). Neal reported highly precise results for the colorimetric assay of titanium both on alloys and pure titanium metal, based on the peroxide method with 1-cm. cells and absorbencies between 2.5 and 3.0 (*132*). A coefficient of variation of only 0.03% was obtained. Factors influencing precision and accuracy were discussed in detail.

Bagshawe and Truman recommended the molybdenum blue method for determining silicon in titanium powders as well as in other metallurgical materials following preliminary ignition and fusion with sodium carbonate (*5*). The method was applied successfully to carbides of titanium, molybdenum, tungsten, and various oxides.

Mikula and Codell determined aluminum in titanium alloys in the range 0.005 to 10% by a polarographic method based on reduction of an aluminum–azo dye complex in conjunction with cupferron and mercury cathode preliminary separations (*115*).

Norwitz, and Codell found the diphenylcarbazide method superior to the titrimetric method for 0.02 to 4% chromium with respect to the time required and freedom from interference by the usual elements occurring in commercial titanium alloys (*135*).

Norwitz, Codell, and Verderame reviewed the difficult problem of determining niobium in titanium alloys and recommended the thiocyanate method with two tannic acid separations (*137*). Interference by other elements was discussed with particular attention to tantalum and tungsten. The method was designed for determining 0.05 to 10% niobium. The same authors also studied the molybdenum blue method for niobium and proposed certain improvements (*134*). An indirect method for determining 0.001 to 0.2% chloride in titanium sponge was based on precipitation of silver chloride followed by turbidimetric measurement of silver as the sulfide (*30*). Tin in titanium was determined iodometrically by Norwitz and Codell in the range 0.05 to 5.0% (*136*). The nitric acid method for the colorimetric determination of carbon in titanium was recommended for samples containing up to 0.7% carbon (*32*). No interference is encountered by the elements normally present in commercial titanium.

Albrecht and Mallett reviewed the determination of oxygen in titanium by vacuum fusion and discussed sample size, ratio of sample weight to graphite, use of a crucible cover, and other variations in procedure (*3*).

An isotope dilution method for determining oxygen in titanium involving exchange with oxygen-18 isotope in a master alloy was proposed by Kirshenbaum and coworkers (*90*). Mixtures of titanium, niobium, and tantalum oxides were analyzed by Beydon and Fisher using radiochemical methods (*11*). Small amounts of tantalum were determined by measuring the activity of tantalum-182 following activation by slow neutrons. Niobium was determined by isotope dilution, adding niobium-95, and precipitating with tannic acid. Titanium was determined colorimetrically.

An x-ray fluorescence method for analysis of binary mixtures of zirconium and hafnium oxides was developed by Mortimore and Romans (*130*). Concentrations from 0.5 to 99.5% of either constituent can be determined in 5 to 10 minutes, including preparation of sample and final calculations. Samples are prepared by briquetting with corn starch to form 1-inch wafers. Two working curves are used to cover the entire range of concentrations. Interference resulting from addition of nickel, iron, tin, titanium, strontium, and rubidium was determined.

Mortimore and Noble developed a spectrographic method for determining 0.003 to 0.4% hafnium in zirconium, involving excitation of zirconium oxide samples in a direct current arc (*129*). The sensitivity of this method is unusually good and precision attainable is adequate for most purposes.

Hudgens and Dabagian used radiochemical methods to analyze mixtures of zirconium and hafnium (*80*). Standards containing known amounts of zirconium were sealed in quartz capsules and subjected to neutron irradiation. Inactive niobium was then added to the reference and unknown samples, followed by separation of Nb_2O_5. Zirconium was determined by measurement of the decay of zirconium-95, and the amount of zirconium-94 in the sample was calculated from the niobium-95 activity. Nonradioactive niobium does not interfere, and interference by uranium can be eliminated by separating barium-140 and calculating the amount of fission niobium present using fission yield data.

Freund and Miner developed an excellent ion exchange method for the separation of small amounts of aluminum from zirconium applicable to samples containing from 0.001 to 3.8% (*52*). The most serious source of interference was found to be iron, which passes through the column and accompanies aluminum, requiring a subsequent cupferron separation. Separations are also required with appreciable amounts of tin and vanadium. Aluminum in the eluate is determined by conventional methods employing 8-quinolinol and aluminon.

Beaumont studied the determination of titanium and iron in zirconium using Tiron (*6*). Of the elements which interfere, copper is the only one likely to be present, in which case, separation by a dithizone extraction is recommended. Methods for the determination of chromium by the persulfate method and tin by the iodometric method following dissolution of the sample in sulfuric and fluoboric acids were reported (*185*).

Read and Zopatti discussed the volatilization method for oxide based on reaction with hydrogen chloride at 450° C. and determination of zirconium in the residue (*147*). Errors of ±5% of the amount present were reported. Their apparatus was modified by Tighe and coworkers to provide magnetic manipulation of the sample and reverse flushing with argon (*181*).

McGeary investigated the hot extraction method for determining hydrogen in zirconium and developed apparatus capable of analyzing five samples per hour, hydrogen being calculated from the pressure rise at constant volume (*104*).

Rodgers and Harter developed a rapid method for determining nitrogen in zirconium based on Beeghly's micro-Kjeldahl steam-distillation method originally designed for steel (*155*). The final measurement is performed colorimetrically using Nessler's reagent and as many as 160 determinations can be carried out in an 8-hour day by two operators with five stills. Standard deviations were of the order ±0.001 at concentrations of 0.003 to 0.02% nitrogen.

NIOBIUM AND TANTALUM

The determination of niobium and tantalum in columbite and euxenite by the dry chlorination method was studied by Treadwell and coworkers (*183*). This determination requires 8 hours' heating at 500° C. with a gas mixture of hydrogen chloride and carbon tetrachloride. Niobium and tantalum are precipitated as the hydroxides after titanium tetrachloride has been separated from the more volatile chlorides of niobium, tantalum, uranium, and iron. The titrimetric determination of niobium in the presence of tantalum was discussed and a procedure recommended.

Hastings and McClarity investigated the determination of small amounts of niobium in tantalum metal based on measurement of the thiocyanate complex of niobium extracted with ether (*74*). The method is reported to give good reproducibility for concentrations of niobium between 0.01 and 0.3%.

Schäfer, Pietruck, and Grözinger studied methods for determining tungsten in niobium-tantalum materials using chlorination with carbon tetrachloride in a closed tube (*158*). Mixtures of chlorinated products containing niobium pentachloride and tantalum pentachloride are extracted with carbon tetrachloride to remove tungsten hexachloride. Tungsten is determined colorimetrically by measurement of the blue color of the hexachloride. Separation of niobium and tantalum by precipitation with sodium chloride is recommended.

Hastings, McClarity, and Broderick, in the analysis of tantalum and tantalum oxide, determined 0.002 to 0.03% iron with *o*-phenanthroline, 0.000 to 0.016% copper with sodium diethyldithiocarbamate, and from 0.004 to 0.07% titanium with hydrogen peroxide (*75*). These methods avoid tedious separations of the metals and contamination from use of large quantities of reagents. Results are satisfactory for assignment of values to spectrographic standards.

Kohn applied radioactivation methods to the determination of tantalum in niobium alloys and minerals without any chemical separations (*93*).

Torrisi, Kernahan, and Fryxell developed a low-pressure combustion method for determining carbon in tantalum based on the apparatus of Murray and Niedrach for carbon in steel (*182*). A multiple sample holder suitable for nonmagnetic samples was developed. The method is applicable to samples containing 0.002 to 0.007% carbon using a 0.5-gram sample. Combustion is carried out for 5 minutes at 1100° C.

Kral and Kysil investigated the polarographic determination of tin in tungsten metal, carbide, and ore (*96*). Mallett and Griffith discussed vacuum fusion apparatus for determining nitrogen and oxygen in molybdenum, capable of determining these gases to within ±1 p.p.m. (*105*). Their studies included effects of surface oxidation.

BERYLLIUM

Analytical chemistry of beryllium was reviewed in detail by Vinci (*187*). Methods of detection, separation, and determination of beryllium were described in addition to methods for determining oxide, carbide, and various metals in beryllium alloys. This is the best source of information on beryllium analysis in the recent literature. Brewer discussed the determination of beryllium in beryl and investigated the Pribil and Kucharsky method based on precipitation with ammonia in the presence of ethylenediaminetetraacetic acid, which holds other elements in solution (*17*). This method appears to offer advantages over the longer method involving precipitation with 8-quinolinol. Hure, Kremer, and le Berquier employed the same masking reagent in the determination of beryllium in beryl based on precipitation of the phosphate (*81*). Catoggio published an extensive review on the chemical analysis of beryl and also proposed a procedure for determining beryllium based on precipitation in the presence of ethylenediaminetetraacetic acid followed by reprecipitation or titration with standard sodium hydroxide (*26*).

Bergholz discussed the analysis of mixtures of metallic beryllium and beryllium carbide based on evolution of hydrogen and methane and combustion of the gases with subsequent measurement of the water and carbon dioxide produced (*8*). The sample was treated with potassium hydroxide to dissolve the metal and hydrolyze the carbide. Free metal can be determined satisfactorily by using hydrochloric acid instead of potassium hydroxide, although this gives low results for carbide.

Read, Funston, and Bridges also described procedures for analysis of beryllium carbide mixtures containing free metal (*148*). They employed an acid hydrolysis of the carbide. Improvements in the phosphate method for determining beryllium were proposed.

Osmond and Smales employed a radioactivation method for determining oxygen in beryllium metal powder based on conversion of the oxygen to fluorine-18, which is separated chemically, after which its activity is compared with that of standards similarly treated (*139*).

SODIUM AND CALCIUM

White, Ross, and Rowan proposed the determination of oxide in sodium based on reaction between sodium and butyl bromide in hexane (*192*). After the reaction between sodium and the bromide is completed, sodium oxide is titrated. With large samples, oxygen below 0.02% can be determined and the standard deviation obtainable is 0.003 to 0.005%. Other impurities can be determined on the same sample.

Pepkowitz, Judd, and Downer investigated the amalgam method for determining oxide in sodium and suggested improvements over their earlier method (*143*). Lithium and potassium can also be analyzed by the same method.

For the determination of trace amounts of boron in sodium metal, Rynasiewicz, Sleeper, and Ryan used a colorimetric method with curcumin reagent (*157*). An extraction with alcohol was found to give satisfactory recoveries of boron, eliminating the necessity of distilling as methyl borate which would otherwise be required. This separation should be useful also for the determination of boron in other materials. Polarographic methods for determining manganese, copper, chromium, iron, lead, and cadmium in calcium metal were reported by Reynolds and coworkers (*150, 151*).

Hering employed ion exchange in the separation of lithium from calcium at concentrations as low as 1 p.p.m. (*77*).

URANIUM

Interest in uranium-bearing materials has stimulated investigation and development of methods for determining this element, many of which have been published. In Rodden's review, covering the period 1949 to 1953, methods of separation together with gravimetric, titrimetric, photometric, spectrographic, x-ray, and various electrometric procedures are described applicable over the range 10^{-9} to 100% (*153*). This is the latest comprehensive review of the subject with 79 references, about half of which cover foreign journals.

Grimaldi and coworkers published a collection of methods for the analysis of uranium- and thorium-bearing materials, discussing general principles together with the procedures for determining trace concentrations (*67*). Use of zinc amalgam for reducing uranium and methods for extraction of thorium by organic solvents are discussed in detail.

Eichholz, Hillborn, and McMahon described various physical methods for assaying uranium and thorium ores based on simultaneous measurement of beta and gamma activities (*42*). Equipment used in this determination is discussed together with mathematical equations for determining the concentrations, taking into account the equilibrium condition of uranium in the samples. Grimaldi, May, and Fletcher prepared a manual for the determination of uranium by fluorimetric methods involving fusion of the sample with a fluoride-carbonate mixture (*66*). Methods of eliminating interference by such elements as cobalt, chromium, nickel, manganese, cerium, and thorium are discussed, and detailed drawings of various fluorimeters in use are shown. In the photometric method for uranium based on fluorescence, Zimmerman, Rabbitts, and Kornelsen recommended making a large number of measurements on replicate samples in order to give a good statistical average (*200*). Notwithstanding the large number of fluorescence measurements, the procedure is stated to be more rapid than other recommended methods and capable of giving average deviations, among laboratories, of less than 1% of the amount present.

Silverman, Moudy, and Hawley described a colorimetric method for determining uranium in the presence of large amounts of iron using 8-quinolinol (*167*). Iron, which would otherwise interfere, is converted to ferrocyanide prior to extraction of the uranium complex.

Milner and Phennah determined titanium in uranium-titanium alloys by the peroxide method in the range 5 to 75% titanium (*121*). The differential method of color comparison between the sample and the standard was employed and theoretical principles underlying the method were discussed in detail. For the determination of uranium in these alloys, a preliminary separation of titanium by extraction of the cupferrate in chloroform was used, followed by removal of residual uranium in the extract by precipitation with tannic acid and subsequent determination colorimetrically as the thiocyanate (*122*). The major portion of uranium was then determined gravimetrically with tannic acid in the aqueous layer. A differential method for determining uranium by the thiocyanate method was also reported by Crouthamel and Hubbard (*37*).

Zirconium in binary alloys with uranium was determined titrimetrically by adding excess ethylenediaminetetraacetic acid followed by back-titration with a standard ferric iron solution using salicylic acid indicator (*123*). This procedure may be used in conjunction with a preliminary separation of zirconium by the usual methods such as precipitation with mandelic acid from a perchloric acid solution of the alloy. This procedure may also be used with a cupferron extraction to remove zirconium prior to the mandelic acid precipitation to avoid interference by impurities such as iron, vanadium, and niobium (*124*). Traces of uranium in zirconium and zirconium in uranium were determined colorimetrically by the thiocyanate and alizarin methods. Milner and Macara developed procedures for the analysis of cobalt-uranium alloys determining cobalt by potentiometric titration with potassium ferrocyanide, silicon by the gravimetric method, and uranium gravimetrically as ammonium diuranate (*120*).

Milner and Wood investigated the problem of separating tantalum and niobium from uranium based on the solubility of the tantalum and niobium fluorides in methylethylketone (*126*). Following the extraction, tantalum and niobium are precipitated by tannic acid. Small amounts of tantalum and niobium in U_3O_8 were determined colorimetrically by the pyrogallol and peroxide methods, uranium being determined by precipitation as ammonium diuranate. These procedures apply to alloys containing up to 25% tantalum and niobium.

Milner and Wood determined gallium in gallium-uranium alloys by extraction with ether followed by precipitation and weighing of gallium camphorate (*125*). This method is applicable to the determination of gallium from 1 to 99%. Uranium was determined colorimetrically in the gallium extract to establish the correction to be added to uranium determined gravimetrically in the aqueous layer. In studying the conditions for extraction of gallium, it was found that the chloride extracted more readily than either the bromide or iodide (*127*). Diethyl ether was preferred as solvent. For determining less than 3 mg. of extracted gallium, titration with ferrocyanide using 3,3′-dimethylnaphthidine indicator was recommended as an improvement over the camphoric acid precipitation.

Haslam, Russell, and Wilkinson determined 0 to 0.02% nickel in uranium colorimetrically with dimethylglyoxime and 0 to 1% manganese titrimetrically following oxidation to permanganate (*73*). Dizdar determined trace amounts of cadmium in uranium polarographically and spectrographically after a preliminary separation by ion exchange (*40*).

SILVER, GOLD, AND PLATINUM

Broadbank and Winram investigated methods for separation of silver, copper, cadmium, and zinc in silver solder by electrodeposition (*20*). These metals are separated by controlling the potential on the electrodes using a perchloric acid medium. Zinc is finally deposited from alkaline solution.

Watanabe studied the fire assay of gold and silver ores containing tellurium and showed that losses of these metals in cupellation are not incurred if sufficient lead is present (*189*). Linhart determined 0.005% gold in silver polarographically as the hydroxide complex with an error of the order of 2% (*98*). Palladium was also determined polarographically in alloys of gold and platinum (*97*). Cadmium interferes and was separated as the carbonate.

A potentiometric method of titrating gold in gold bullion was based on reaction with hydroquinone or ferrous ammonium sulfate (*156*). Platinum and iridium were also titrated potentiometrically and palladium was determined gravimetrically after precipitation with dimethylglyoxime. A direct photometric method for determining silver in native gold was based on measurement of reflectance of the sample as received using a blue filter (*2*). Reflectance was found to vary between 36 and 87% going from pure gold to pure silver. A simultaneous method for determining palladium and platinum in silver products was based on extraction of the colored product formed by reduction with chlorostannous acid, and comparison with suitable standards (*92*). Selenium and tellurium require a separation.

A gravimetric method for determining palladium in jewelers' alloys was described by De Hovre and based on precipitation of palladium with a hydrobromic acid solution of *p*-aminoacetophenone (*39*). The precipitate is ignited to the metal. Sindler determined sulfur in noble metals by measurement of thiocyanate following reaction with potassium cyanide (*171*).

MISCELLANEOUS

Problems encountered in the analysis of metallurgical chrome ore were reviewed by Hartford including a statistical treatment of a large number of ore analyses over a period of 8 years (*71*). Uncertainties in the determination of chromic oxide, iron, silica, aluminum, and magnesium were pointed out, and refinements in the procedures in use were suggested. Establishment of an industry standard for metallurgical chrome ore was discussed in detail. Zivanovic described methods for analysis of chromite ores, including potentiometric titration of chromium and determination of iron and silica (*201*).

Rodewald determined lead in chromic oxide gravimetrically as the sulfate or chromate and recommended the spectrographic method for lead in chromium metal (*154*). Horton and Brady developed a vacuum fusion method for determining oxygen in chromium employing a 3 to 1 iron-tin bath operated at 1600° C. (*78*). Limitations of the vacuum fusion method and effects of certain variations of the procedure were discussed. Recovery of oxygen was found to have dropped to about 70% when the bath concentrations had reached 5% chromium.

Margerum, Sprain, and Banks developed a colorimetric method for determining aluminum in thorium based on extraction of aluminum with 8-quinolinol and measurement of the extracted aluminum complex (*110*). Extraction of thorium was prevented by use of a suitable masking agent. From 2 to 120 mg. of aluminum may be determined to within 0.5 mg. Any material convertible to the nitrate may be analyzed by the proposed method.

Silverman and Trego determined up to 0.6% iron in thorium metal and its compounds by direct measurement of the absorption of ferric chloride, no separations being required (*169*).

Gibson studied the molybdenum blue method for determining silicon, applying it to bismuth oxide and metal (*55*). Results in the range 5 to 35 p.p.m. were reported to be within ±1.5 p.p.m. Long and Sackman determined metallic bismuth in the presence of the oxide by shaking with excess silver nitrate, filtering, and back-titrating the excess silver by the Mohr method (*99*).

Haupt and Olbrich prepared spectrographic standards for determining lead and thallium in high-purity metal based on the polarographic determination of these metals (*76*). Lead was first separated by precipitation with ferrous hydroxide and thallium by an ammonia precipitation.

Kojima determined traces of lead in cadmium sulfate electrolyte by the dithizone method following a preliminary separation of lead and strontium with a cation exchange resin (*94*).

Luke and Campbell proposed colorimetric methods for the determination of 0.1 to 1 p.p.m. of arsenic, phosphorus, antimony, and copper in germanium and germanium dioxide (*102*). The method for arsenic involves separation from germanium and other interfering metals by extraction with diethylammonium diethyldithiocarbamate in chloroform and measurement as molybdenum blue. Arsenic in silicon can be determined by a similar procedure.

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Food

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THIS review covers some 2 years that have passed since the previous one was completed (*190*), and includes papers published during this time.

MOISTURE

Research on moisture determination by nuclear magnetic resonance has been continued, and a recent paper (*263*) reports that this method has shown promise for rapid routine use. One important advantage is that it does not require essential modification of the test specimen. The method is effective in moisture ranges from a few per cent to as high as 100%.

Moisture determinations with the Karl Fischer reagent have been critically examined and discussed in three papers (*57, 152, 281*). Efforts have been made to substitute other reagents for methanol, and formamide has been found to be more versatile than methanol for moisture in such foods as beans, cake mix, dried whole eggs, egg noodles, rolled oats, rice, and wheat (*177*). Another modification makes use of a solution of bromine and sulfur dioxide in anhydrous chloroform instead of methanol (*14*). In the determination of moisture in honey using different methods, the Karl Fischer technique gave the highest values, while the refractive index method of the AOAC gave the lowest value (*1*).

A method has been proposed (*290*) for estimating moisture in sugar-containing products, such as honey, jams, and fruit juices, based on the property of absolute alcohol to form an azeotropic mixture with water of the sample, and upon the use of a special colloidal silicate which dries easily. In the case of dry sugar, it was found (*94*) that drying the sugar at 130° C. for 45 to 60 minutes gave results identical with those obtained by drying at lower temperatures for 4 hours or longer.

Moisture determinations in cheese have been carried out by placing the cheese between two previously weighed sheets of unlacquered aluminum 15 × 19 × 0.0015 cm. (*258*). This is somewhat similar to the glass rod technique of the AOAC (*48*), in which the cheese is smeared on a glass rod. This method results in an improvement in the precision of the vacuum oven method for determination of moisture in both process cheese and cheese food, in that there is less burning of the sample. It has been reported (*163*) that loss of moisture in cheese is most rapid when final drying in a water or vacuum oven is preceded by heating in a water bath for 1 hour.

An apparatus for determining moisture in grains and flour by use of electric conductivity has been patented in Japan (*136*) Application of the old toy used to predict the weather (not too accurately) has been utilized to measure moisture in milled products (*230*). Dried filter paper is impregnated with cobalt chloride, which changes from blue to red when exposed to moisture. An infrared method has been proposed for measuring moisture in starch (*294*). A 10-gram sample is placed in an aluminum dish and irradiated with a 250-watt infrared lamp at 10 cm. After 15 minutes the sample is cooled in a desiccator and weighed.

Several methods have been suggested for determining moisture in fats and oils. A procedure using calcium carbide (*58*) has been suggested, as well as a modification of the Roberts and Levens technique using the Karl Fischer reagent (*193*). The thermal balance Buhler moisture apparatus allows weighing of oilseed samples without removal from the oven (*87*). At 115° to 125° C. results are obtained in 5 to 10 minutes; they are slightly higher, however, than the official oven method. It has been suggested (*72*) that good results can be obtained by drying the fat at 70° to 75° C. in a desiccator which is being continuously evacuated and flushed with carbon dioxide.

A quick method for determining water in sausages involves the use of equations with correction factors (*22*).

PROTEINS AND AMINO ACIDS

The Kjeldahl method is still the subject of considerable research. No general Kjeldahl method seems to be satisfactory for all types of nitrogen-containing compounds (*25*). Conditions of digestion as related to temperature, catalysts, and oxidizing agents have been studied (*182, 241*). A modified method has been reported (*226*) in which digestion should not take more than 20 minutes for easily digested materials. Good results have been obtained in destroying organic matter with a mixture o sulfuric acid, potassium peroxydisulfate, and hydrogen peroxide (*300*). In regard to catalysts, a study (*108*) has shown that best results are obtained by using a mixture containing selenium and mercuric oxide. The addition of hydrogen peroxide is recommended for high-fat materials. In a semimicromethod (*107*), sodium hydroxide and sodium thiosulfate are used in place of sodium monosulfide. Where the conventional sodium sulfate is used, it has been shown that 18 grams of the material, rather than the usual 10 grams, gives better results (*26*).

Of interest are two papers dealing with amino acid determination in foods by chromatography (*96, 257*). One method uses a Dowex 50 column by means of which nearly all the common amino acids can be determined (*257*). The other method (*96*) uses a circular paper chromatography technique, and is said to be ideally suited to the study of amino acid changes brought about by the processing of foods. Also of interest is a review article on chromatographic ion exchange separation of amino acids, purine and pyrimidine bases, nucleotides, sugar derivatives, carboxylic acids, and proteins (*46*).

Circular paper chromatography has many advantages in the small laboratory, owing to the simplicity of the apparatus and techniques employed. A paper dealing with a systematic study of the physical factors influencing the R_f for a simple amino acid system is of interest (*248*). The R_f value of some 21 amino acids and 5 peptides has been determined by circular paper chromatography (*236*). Separation of glycine, serine, and aspartic acid from each other, and of glutamic acid and threonine, has been achieved by two-dimensional chromatography (*98*). Lack of reproducibility, irregularly shaped spots, discoloration of the paper, and incomplete separation have been overcome by using a solvent system of butanol, acetic acid, water, cresol, and phenol with a borate buffer (*172*). Isatin as a color reagent in amino acid circular chromatography has been described in two papers (*247, 270*).

A paper describing a modified procedure for the photometric determinations of amino acids with ninhydrin reveals that 10 of the naturally occurring amino acids give theoretical yields of color at room temperature (*292*). At higher temperatures all amino acids except tryptophan and lysine react quantitatively.

A rapid, simple quantitative biuret test for milk serum proteins has been reported (*145*), but inasmuch as the color response of casein is different from that of serum proteins, mixtures of these proteins cannot be analyzed by the biuret test. Formol titration has also been suggested (*149*) as a rapid and dependable method for determining protein in milk.

Albumins and globulins in wheat flour can be estimated quantitatively by a method developed from the characteristic tryptophan and amide nitrogen contents of the albumin, globulin, and gliadin protein of the flour (*224*). Results are reliable to within approximately ±10%. Glutamic acid in wheat gluten is estimated by determining total dicarboxylic acids (glutamic and aspartic) by adsorption on Amberlite 1R-4B at pH 3 to 4 with subsequent elution with 1*N* hydrochloric acid (*203*).

A method has been proposed for determining ammonia, amide, nitrite, and nitrate nitrogen in plant extracts by using a single portion of the extract (*297*), and a systematic procedure has been reported (*222*) whereby water-soluble extractives of plant and animal tissues are separated into groups for identification or quantitative estimation by paper chromatography.

METALLIC IONS

A critical review of existing procedures for the preparation of biological material for the determination of trace metals has been presented (*195*). Spectrochemical methods have been suggested in five recent papers (*109, 118, 191, 229, 309*). In one procedure (*309*) the minor elements, such as cobalt, zinc, and copper, are first separated in the form of dithizonates from the plant material. Spectrochemical analysis of plant material using spark excitation has the important advantage that all major inorganic elements as well as the usual minor elements can be determined simultaneously from one exposure (*191*).

Quite apart from spectrochemical technique for determining trace elements is a suggested microbiological method (*208*) employing such fungi as *Aspergillus niger* and *Penicillium glaucum* for estimating iron, copper, zinc, manganese, and molybdenum in biological materials.

Although organic insecticides have largely replaced arsenical sprays in the fruit industry, appreciable amounts of lead arsenate are still used for apples and other deciduous fruits. The bromide distillation method of Magnuson and Watson has been adapted for the determination of arsenic spray residues on fruits and vegetables (*13*). Interference of tin (in the case of canned foods) is overcome by extracting tin with cupferron and chloroform before distillation.

A colorimetric method more accurate than the Gutzeit test has been suggested for the determination of arsenic in gelatin (*71*), and estimation of arsenic in eggs involves distilling to obtain arsine and then reading the developed color in a spectrophotometer (*77*).

Colorimetric determination of copper in plant material has been suggested by means of both the dimethyldithiocarbamate complex (*85*) and with sodium diethyldithiocarbamate (*43*), which is said to be one of the most sensitive chemical tests for copper.

Copper in beers and ales acts to accelerate oxidation changes, adversely affecting flavor and shelf life. A colorimetric procedure for copper determination which requires no ashing of the sample and gives results in about an hour has been suggested (*280*). Reagent used is zinc dibenzyldithiocarbamate which gives a brownish copper complex subsequently measured in a photometer.

A method for estimating lead in foodstuffs is based on the extraction of the dissolved ash with a solution of diethylammonium-diethylcarbamate in chloroform at pH 7.0 (*175*). The method is more accurate than the usual sulfide technique and more convenient than the absorptiometric method with dithizone solutions. Ashing of the material must, of course, be carried out with care and ignition of the sample in a nickel oxygen-filled bomb has proved satisfactory for burning of foodstuffs prior to lead determination by any suitable technique (*78*).

A titration method for calcium and magnesium in milk, in which interfering phosphates are first removed by a simple anion exchange technique, has been suggested (*142*), while a method for determining calcium in plants is based on measuring the resulting turbidity when a reagent containing ammonium oxalate, citric acid, ethanol, and butanol is added to a buffered aqueous extract of the material (*135*). From 0.05 to 0.50 mg. of calcium can be determined. A micromethod (*115*) which can estimate as little as 4 γ of calcium is based on the precipitation of calcium molybdate and subsequent formation of molybdenum thiocyanate, which is determined photometrically.

In the determination of phosphates in biological materials, a micromethod (range, from 1 to 70 γ of phosphorus) makes use of an absorptiometric technique based on the conversion of phosphorus to phosphomolybdic acid and reduction of molybdenum blue by stannous chloride (*117*). Procedures for determining total, inorganic, acid-soluble, phosphatide, and phytin phosphorus in plant materials are described in detail in another paper (*231*).

A photocolorimetric method has been proposed for tin in canned foods (*67*), while a suggested procedure for zinc is based on the fact that diantipyrylmethylmethane produces a white turbidity in the presence of zinc (*103*). The turbidity is measured nephelometrically. Lead and ferric iron interfere and must be removed prior to analysis.

Sulfur in plant materials is estimated turbidimetrically in amounts ranging from 0.01 to 2.5%, using as little as 0.1 to 0.3 gram of material (*275*), while a flame photometric method for potassium gives good agreement with results obtained by the platinic chloride procedure (*146*).

Methods generally used for determining fluoride in vegetation give low results with samples high in silica. A modified procedure (*244*) eliminates the need of a double distillation and evaporation by using alkali fusion of the ash and collection of 500 ml. of distillate.

Estimation of oxygen in fruit juices is of importance in studies on quality changes in juice products during storage. An apparatus and polarographic method are described which should be useful to those concerned with this field of food research (*240*). A preashing apparatus, used for highly foaming sugar solutions, is also of interest (*52*, *53*).

FATS AND OILS

Increased production of vegetable oil "ice cream," which amounted to some 22,500,000 gallons in 1953, has aroused interest in methods for the detection of vegetable and animal fats in dairy products. One method suggests a rapid chromatographic determination of molar concentrations of butyric acid in the fat of dairy products (*112*). The amount of fat substituted can, however, be estimated only semiquantitatively using this method. Another procedure measures the refractive index of the alcohol-insoluble triglycerides to determine foreign fat addition (*17*).

Two methods have been offered for improving peroxide number determinations. One suggests adding a smaller quantity of potassium iodide and carrying out the determination in the same container from beginning to end (*106*). The other, of particular value for dairy products, when the thiocyanate method for peroxide number is used, utilizes a nonionic detergent to break the emulsion (*278*). It has been reported (*99*) that the thiocyanate method shows good correlation with values obtained by the Swift stability procedure.

The thiobarbituric acid test for oxidative rancidity, which has been used in the past for milk fats, has been modified for use with butter, Cheddar cheese, and powdered whole milk (*18*) and for frozen pork (*293*).

The Babcock test for butterfat has been reviewed (*119*, *123*). The Bureau of Dairy Industry's detergent test for butterfat (*246*), in a limited number of comparisons, showed close agreement with the Roese-Gottlieb and Babcock tests. Another paper reports results 10 to 40% below those of the Gerber method when BDI technique was used (*2*). It has also been suggested that a detergent be added to the sulfuric acid (9 grams of a 50% quaternary ammonium compound to each 1000 ml. of sulfuric acid) and glymol before measuring the fat volume (*303*). In measuring the fat content of half-and-half, which contains 8 to 13% fat, the usual Babcock technique may result in charred fat. It is suggested that ice cream test bottles be used with the addition to the sample of 9 ml. of a saturated solution of trisodium citrate and 2 ml. of propyl alcohol prior to the addition of sulfuric acid (*192*). The use of hydrochloric acid in determining butterfat gives slightly lower results than those obtained by the Roese-Gottlieb method (*206*). It has been reported that results from the Schain butterfat test are statistically closer to those of the ether extract method than is the case with the Babcock test (*148*). A modified butyrometer method for fat in cream compares well with the Roese-Gottlieb procedure (*132*).

An apparatus previously described for determining oil in soybeans can also be used for estimating oil in flaxseed (*134*). Two other methods of estimating fats in oil-bearing seeds have been suggested (*9*). One is a Soxhlet extraction technique, while the other depends upon the uptake of oil by sodium sulfate, which is subsequently treated with a solvent. An improvement in the method of determining the oil content of olives pertains to the introduction of a Waring Blendor into the process (*147*). Methods are also given for the detection of the addition of other oils to peanut oil (*298*) and determination of total fat, milk fat, and coconut oil in chocolate products (*107*, *254*). By the semimicro butyric acid number, milk fat in chocolate products can be easily determined even in the presence of coconut oil as high as 3% of the mixtures of fat (*254*).

A few minor changes have been recommended for the American Oil Chemists' Society's tentative spectrophotometric method of the analyses of fats and oils (*277*), and a study of spectrophotometric methods for polyunsaturated fatty acids in cottonseed oil makes a comparison with chemical methods (*215*).

A wet-ash colorimetric procedure has been offered for estimating lipide phosphoric acid in eggs and egg noodles (*61*) and a simplified procedure for determining the approximate lecithin-nitrogen and phosphoric acid content of macaroni serves as the basis for estimating the egg content of the product (*122*).

A spectrophotometric method for grading green soybean oil correlates spectral absorption and visually determines green grades of oil as a suggested basis for a simple, rapid spectrophotometric method for grading (*194*).

The induction period of butter is measured (*312*) by spreading on a glass plate and exposing the sample at room temperature to normal sunlight or ultraviolet. Periodic weighings and determination of the aldehyde content, as well as observations of color and odor, are made. The appearance of aldehyde corresponds to the point of increase in weight—i.e., end of the induction period. In determining aldehyde values of slightly oxidized butterfat by Schibsted's method, interference of butterfat color is eliminated if measurement is carried out in a photoelectric colorimeter at 510 mμ (*69*).

Fatty acids are determined by running paper chromatograms in butanol—ammonium hydroxide mixtures and then spraying with a mixture of methyl red, and bromothymol blue indicators in formalin (*70*), and by a potentiometric method in which a common glass electrode serves as a titration vessel for titrating with potassium hydroxide, using potassium acid phthalate and benzoic acid standards (*101*).

The methoxyl content of lard involves saponification, recovery of methanol by distillation, oxidation, and reaction with chromatropic acid (*6*). Absorbance (optical density) of the colored solution at 5700 A. is proportional to the methoxyl content.

A shortening consistometer (*44*) provides a simple and rapid means of obtaining numerical measurement of the consistency of shortenings, margarine, and butter, and is particularly suitable for production control purposes.

A report dealing with the estimation of ergosterol in yeast is also of interest (*264*).

ENZYMES

Some batches of filter papers used for the phosphatase test for pasteurized milk contain a reacting substance in sufficient quantities to cause a properly pasteurized milk filtered through such a paper apparently to fail to satisfy the test (*47*). Details of a modified phosphate procedure are given in another paper (*252*). The phosphatase test has also been suggested as a means of control in heat-treated meat products (*267*).

Pink center development in frozen Brussels sprouts, probably due to inadequate blanching, has caused some concern to freezers of this vegetable. It has been suggested (*189*) that instead of using the peroxidase test as proof of adequacy of blanching, a dilute solution of hydrogen peroxide be added to Brussels sprouts halves to obtain an orange color. Development of this color roughly parallels peroxidase content. Another report of interest is a method of measuring enzyme activity in frozen foods (*185*) by a spectrophotometric technique.

Paper chromatographic behavior of eight enzymes has been described (*143*). Solvent systems used were mixtures of aqueous buffers and organic liquids, among which acetone and methanol were found most suitable.

A review of methods (*24*) suitable for evaluation of protease activity in doughs will be of interest to cereal chemists, and it has been suggested that activity of proteolytic enzymes can be estimated by the biuret reaction (*268*).

A method (*156*) for estimating lipase in cereals involves the use of a butterfat emulsion as a substrate buffered at pH 7.9 with either phosphate or borate. The material is maintained at 36° C. and electrometrically titrated with standard alkali.

CARBOHYDRATES

The green color produced when carbohydrates are heated with anthrone in acid solution was first used about 8 years ago to estimate carbohydrates. A recent paper (*310*) reports that the anthrone method for soluble sugars in plant extracts yields results comparable with those obtained with the copper reagent, but includes the sugars of stable glycosides which may constitute a large proportion of the soluble carbohydrate in some plant tissues. Use of the anthrone reagent has been extended to the quantitative determination of pentoses, by employing a simple modification of operating techniques (*28*). Hexoses will interfere, if present. Pentoses will also interfere with determination of hexoses, depending largely on the composition of the anthrone reagent. Methods have been described (*144*) for reducing this interference.

A monograph with 120 references dealing with crude fiber determination suggests slight modifications in the present method (*128*). It has also been pointed out that the percentage figure of crude fiber, especially when calculated on a dry basis, is a far more useful criterion in estimating food value than is nitrogen-free extract (*219*).

Two variations in estimating sugar by Fehling's solution are of interest. In one (*296*) the filtrate obtained from the cuprous oxide precipitate is examined colorimetrically using a comparison solution of invert sugar. In the second (*129*) the cuprous oxide is dissolved in hot $4M$ nitric acid and diluted, and an aliquot is mixed with $1M$ ethylenediamine to obtain the copper-ethylenediamine complex, which is examined spectrophotometrically at 550 mμ.

Of interest to those concerned with sugar production is a paper giving the characteristic pH values of all types of beet-sugar factory juices, together with temperature coefficients of pH change for each type of juice (*10*). Equally of interest are British specifications for four sizes of graduated flasks for sugar analyses (*29*).

A carbon column adsorption procedure is suggested for the separation of sugars of honey into monosaccharides, disaccharides, and higher sugars (*302*). Caramelization of the sugars in flour when heated with alkali is used to measure total and reducing sugar (*56*). These figures, along with gluten quality, permit estimation of baking quality. A refractometric method has been reported for estimating sugars in ice cream (*271*). The readings obtained must be corrected by means of suitable tables for various brands of ice cream.

Quaternary ammonium and sulfonic acid—type resins have been used in paper chromatography of sugars (*308*); a 0.3% ethanol solution of *p*-aminohippuric acid has been found to be very sensitive and stable, and spots produced by reducing sugars do not fade (*251*). There is also an almost imperceptible background discoloration.

Glucose dehydrogenase has been employed for the direct stoichiometric determination of D-glucose in a variety of corn sirups (*301*). In the presence of oxygen the enzyme oxidizes D-glucose to D-gluconolactone, which is determined quantitatively by titration with standard alkali. Eggs desugared by glucose oxidase and catalase are identified by the presence of gluconic acid, which gives a color reaction with buffered ferric chloride solution (*259*). A reliable and accurate method has been reported (*220*) for the analysis of a mixture of glucose, maltose, and fermentable oligosaccharides, using a modification of a technique published earlier.

By measuring the ammonia expelled from hydrolysis of cyanohydrin, a method has been devised for estimating glucose and other aldose sugars (*49*). When a solution of fructose or sorbose is heated with Folin-Denis reagent and trisodium phosphate, a blue color is produced, and this color is used in determining the amount of sorbose and fructose (*113*).

A method of determining pentoses and hexoses when present together is based on the fact that when such a mixture is heated with orcinol and acid, colors produced can be measured at suitable wave lengths and the results calculated directly from the colorimetric readings by means of a nomogram (*80*).

It has been suggested that lactose in cheese and other dairy products can be determined by a technique based on Somogyi's blood sugar method (*284*); with the anthrone reagent (*79*), results of which agree well with copper reduction methods; and by chromatographic analyses (*131*) involving direct application of milk to the strip and subsequent determination of lactose on the chromatostrip.

A review of methods of testing for nonfat solids in milk is also of interest (*125*).

Pectin in citrus juices is estimated rapidly by a method involving the use of the carbazole–hexuronic acid–sulfuric acid reaction (*68*). Results are reported in terms of anhydrogalacturonic acid (the basic structure of the pectin molecule), and the procedure is suitable for routine control work, a complete analysis taking 3.5 hours. Pectin methods have also been reported (*165, 169, 181, 286*).

A method for estimating water-soluble cellulose derivatives, sometimes used in foods as thickening agents, has been reported by German investigators (*171*).

The MacMichael viscometer method with lactic acid for determining the alkaline viscosity test for soft wheat flours has been modified to an alkaline viscosity test with sodium bicarbonate (*81*). Application of the Corn Industries Viscograph to potato starch has been reported (*65*). Several modifications in design were made while retaining the more important parts of the installation (*64*).

A simple qualitative test for starch sirup in foods has been offered (*15*), and several methods for determining starch in flour and baked goods have been reviewed and compared (*104*).

VITAMINS

Biological, microbiological, chemical, and physicochemical methods for determining vitamins have been reviewed in a recent paper (*211*), and limitations of the design usually employed for microbiological assays have been rather thoroughly discussed (*307*).

Total carotene in tomatoes is estimated by an improved, rapid colorimetric method (*176*), and in another method, vitamins A and E are separated by chromatography on filter paper impregnated with 10% silicone stopcock grease in dimethyl chloride using an ethanenitrile-water system (*31*). The vitamins are located on the strip with an automatic spectrophotometric arrangement which provides a graph of band position *vs.* strip length. A standardization procedure for adsorbants used in chromatographic columns for vitamin A separation has been described (*304*).

A rapid method (*159*) for calculating the Morton-Stubbs correction in vitamin A assays is based on the fact that usually analysis is based on absorbance (A) readings at 310, 325, and 334 mμ. The corrected absorbance is $7(A_{325} - A_{310}) + 4.375\ (A_{310} - A_{334})$ and may be solved with a slide rule. Direct application of the Morton-Stubbs correction to vitamin A samples containing appreciable quantities of tocopherols yields high results for vitamin A potency. The two vitamins must first be separated chromatographically, followed by subsequent quantitative recovery of vitamin A, to which the Morton-Stubbs correction can then be applied (*262*).

It has been suggested that 1,2-dichloroethane can be used in place of chloroform in the preparation of the Carr-Price antimony trichloride reagent (*178*), and this method has been proposed for estimating vitamin A in several dry food products (*35*). Vitamin A is extracted from the food with ether, and the residue left after evaporation of the ether is taken up in chloroform. It has been suggested that the ether extract be stabilized with purified hydroquinone (*202*).

Chemical methods of determining free and phosphorylated thiamine, riboflavin, and ascorbic acid have been discussed (*232*). A method that offers ready separation and independent determination of nicotinic acid and nicotinamide is described, which makes possible the analysis of mixtures containing only one part of one form of the vitamin in the presence of 100 parts of the other (*285*). A gravimetric method for determining choline in eggs is based on its precipitation as reineckate (*105*), and choline and thiamine have been separated quantitatively by chromatography on starch (*124*). The possible errors inherent in the U.S.P. and International Pharmacopoeia thiochrome method for thiamine have been discussed in a French publication (*51*), and estimation of thiamine in bread involves removal of the vitamin by extraction with warm water containing sulfuric and acetic acids (*250*).

A rapid assay method for riboflavin in milk has been reported (*273*) and a chromatographic technique for folic acid has been suggested (*158*). A new microbiological determination of the individual components of the vitamin B_6 group involves extracting them from natural materials by hydrolysis with 0.055N sulfuric acid at 20-pound pressure for 3 hours (*90*). Total B_6 is assayed by using *Saccharomyces carlsbergensis* 4228. Other aliquots are assayed by cation-anion resins, followed by the use of *S. carlsbergensis*.

A modified chemical method for pantothenic acid in wheat consists of extracting pantothenates with methanol instead of water (*237*). In every other respect the method is the same as that of Szalkowski *et al.* A microbiological technique has been suggested for pantothenic acid in foods (*291*). Phosphatase-liver enzymes are used for preparing the sample extracts and ordinary laboratory equipment can be utilized.

Suggested methods for determining vitamin B_{12} have been based on the use *Euglena gracilis* var *bacillaris* (*121, 127, 139*). Response curves obtained with *Lactobacillus leichmannii* using the U.S.P. titrimetric method for B_{12} usually yielded straight lines when log dose was plotted against log response, which makes possible the use of a 6-point (3-dose) design (*183*). Use of *L. lactis* for the plate method and *L. leichmannii* in the acidimetric method has been suggested (*23*). A microbiological method using *L. leichmannii* and measuring response in terms of turbidity has been adopted by the AOAC (*161*). Essentially the same method has been included in the fourth supplement of the U.S. Pharmacopeia (*295*). A rapid spectrophotometric procedure for B_{12} involves extraction of the vitamin with benzyl alcohol or n-propyl alcohol, followed by estimation of the vitamin in the extract by differential spectrophotometric measurements (*83*). A rat-growth method of assay for B_{12} using a highly purified diet with casein has been further studied and improved (*89*).

Use of 2,4-dinitrophenylhydrazine for vitamin C in milk has been suggested (*110*), and a method based on the reduction of picric acid to picranic acid by vitamin C permits a colorimetric estimation of this vitamin (*66*). Cacotheline has been found suitable for the detection of less than 5 γ of vitamin C on paper chromatograms (*289*), and another method involves the reaction of vitamin C with diazotized 4-methoxy-2-nitroaniline in an acid medium, followed by development of a blue color in alkaline solution (*256*). This color, maximum absorbancy at 570 mμ, is compared with standards in a photoelectric colorimeter.

In the chick assay of vitamin D, precision of analyses was significantly greater with selected chicks for uniformity of response (*150*), and improvement in the line test was obtained by giving the bones a preliminary wash in an ether-acetone mixture, thus making for more complete staining with silver nitrate (*266*). Methods have been suggested for determining vitamin D in homogenized milk (*299*) and in various oils containing vitamin A (*255*).

Almost all methods for determining the individual tocopherols in natural products have the disadvantage that the concentration of α-tocopherol, the most biologically active member of the vitamin E group, is estimated by difference. A suggested method involves paper chromatography for quantitative estimation of the individual tocopherols, α-, β-, γ-, and δ- (*30*). A rapid method for vitamin E in foods and fodder is based on Emmerie-Engel's color reaction (*212*), and a modified paper chromatographic technique has also been described (*73*).

A paper giving the R_f values of a number of flavonoid compounds is of interest (*41*).

ACIDS

Two reports describe circular paper chromatography for determination of organic acids (*5, 97*), a technique that in general has been found to be more direct and less time-consuming than other chromatographic methods.

Citric acid is estimated by utilizing the conversion of this acid to pentabromoacetone as recommended in earlier methods, but substituting a direct polarographic measurement for the subsequent petroleum ether extraction, oxidation-reduction treatment, and final titration procedure (*74*).

Microdetermination of citric acid is based on a rapid colorimetric method (*288*). Citric, malic, and tartaric acids are separated by ion exchange chromatography and then determined by oxidation with permanganate (*253*). The method is applicable to fruit, fruit juices, and fruit products. Organic acids in carrot and apple tissue have been determined by paper chromatography (*34*).

Oxalic acid determination in plants is based on esterifying the material in the presence of sulfuric acid, evaporating the ester, and collecting the condensate, which is subsequently saponified; oxalic acid is titrated with potassium permanganate (*168*). The chief difficulty is that distillation, which must be carried out in special apparatus, requires 6 hours.

An ion exchange resins technique for determining organic acids in sugar beets makes it possible to follow the effect of processing variety and other agronomic factors on the organic acid content of the beets (*218*). Nonnitrogenous organic acids in sugar cane are estimated by the application of partition chromatography on silicic acid columns of large capacity (*243*), and partition chromatography has also been recommended for determination of organic acids in sorghum sirup (*184*).

Chromatographic methods have been proposed for estimating volatile acids in cheese (*111, 173*).

COLOR AND TASTE

A rapid spectrophotometric method for measuring the alcohol-extractable color of capsicum spices employs a 10-cm. sample cell and involves transmittance readings at two wave lengths (*188*). Accuracy of the method surpasses that of Lovibond glasses.

Further reports have been issued relative to color grading by the Purdue color ratio meter (*62*), and a photoelectric reflectance meter for measuring the darkening of food purees has been evaluated (*174*). A comparative study of the validities and precisions of three instruments to estimate color grade of Marshall and Northwest strawberries gives correlation data between the instruments (*261*).

Chromatographic methods for coloring matter in foods have been reviewed (*63*). One such method uses glass plates (*233*) on which a thin layer of aluminum oxide is spread. The method permits two-dimensional chromatography. The R_f values of 36 food dyes have been determined by partition filter paper chromatography (*8*). Other chromatographic techniques for determining added colors in foods have also been described (*42, 86, 138, 201, 221*).

Those concerned with taste test methods will find of value a paper on the comparison of the triangular and two-sample taste-test methods (*39*). A sensory taste method has been suggested (*59*) that can be used for estimation of levels of significance for replicated judgment of two or more samples that represent no more than one difference. Of interest is a report on the aroma number (*155*), which is a measure of the potassium bichromate reduction produced by volatile material from 2 ml. of fruit juice as determined under specified conditions in a special apparatus.

Those concerned with quality control will find of interest a paper dealing with a brief discussion of methods used for the quantitative evaluation of fruit products (*45*).

INSECTICIDE RESIDUES

Vapors of perchloroethylene have been found useful in controlling rot-producing molds in strawberries. A method has been suggested (*187*) for determining residual fungicide, involving extraction of perchloroethylene with ether, followed by evaporation in the presence of ethylbenzene, and determination of the chloride with a nephelometric technique. An improved procedure for determining bromine residues has been offered (*287*) and techniques for determination of residues of the herbicide 3-(*p*-chlorophenyl)-1,1-dimethylurea have been suggested (*20, 21, 311*). Parathion residues on tomatoes are determined by removing the residues with alcohol, oxidizing the plant pigments with hydrogen peroxide, and hydrolyzing the parathion to *p*-nitrophenol, which is determined colorimetrically (*36*).

Microquantities of benzene hexachloride can be estimated by an absorptiometric technique (*228*), and residues of the herbicide *N*-1-naphthylphthalamic acid by hydrolysis of the amide to give 1-naphthylamine, which is steam distilled from an alkaline solution (*269*). The amine is coupled with diazotized sulfanilic acid to give a red azo dye which can be determined colorimetrically.

Procedures for the determinations of residual highly chlorinated pesticides such as aldrin and dieldrin have been reported (*4, 55, 197, 198, 216*). Crag herbicide 1 [sodium 2-(2,4-dichlorophenoxy)-ethyl sulfate] is estimated by measuring the intensity of the colored complex formed with methylene blue chloride (*130*), and residues of malathion are determined by rapid decomposition of the material by alkali to form dimethyl dithiophosphoric acid, followed by subsequent determination of this decomposition product by a colorimetric procedure (*214*).

CONTAMINATION AND SPOILAGE

Considerable research has been accomplished on detection of insect infestation of foods. Differentiation of bran and germ from insects and larvae fragments in white flour consists of suspending the specially prepared sample in a dilute crystal violet solution (*164*). By transmitted light, insect fragments appear bright orange-red, while bran and germ particles are stained deep violet. Staining with ruthenium red is suggested for detecting dung and plant fragments in milk (*260*). Internal or hidden insect infestation of grains is detected by means of a low noise level audioamplifier microphone and loudspeaker (*3*). It has also been suggested that insects in stored grains can be identified by their mandible characteristics (*162*), and a recent series of papers dealing with insect contamination in foods is particularly timely (*151, 179, 209*).

Improved techniques have been offered for mold and rot in tomato catsup (*180*) and in tomatoes (*38*). Difficulties in determining rodent hairs and insects in oatmeal have been surmounted (*116*).

Procedures suggested for determining penicillin and other antibiotics in milk include a cup method on agar (*137*), use of a spore suspension of *Bacillus subtilis* (*100*), and utilizing strains of *Streptococcus lactis* having extreme sensitivity to the particular antibiotic being detected (*170*).

Biphenyl is used for controlling decay of citrus during shipment and storage. A method of estimating biphenyl in treated fiberboard cartons is based on using the biphenyl absorption peak at 14.34 microns (*154*). Quaternary ammonium compounds are determined in milk by titration with standard anionic surface-active agents using eosin as an indicator (*92*).

Abnormal quantities of free galacturonic acid in fruit and vegetable products have been proposed as evidence of decomposition by microbial activity. A method for detecting galacturonic acid is based on a modification of Tollen's reaction of uronic acids with naphthoresorcinol (*7*). A direct count method for bacteria in frozen citrus concentrates has been offered (*306*); a rapid procedure for identifying bacteria isolated from off-flavor juice is based in part upon cell morphology (*16*), and rapid colorimetric methods for detecting *Lactobacillus* and *Leuconostoc* are based on estimating diacetyl, one of the end products of bacterial metabolism (*40, 75, 126*). Improved media for the growth and isolation of spoilage organisms from canned foods have also been presented (*84*).

A column chromatographic method is used for the detection of added lemon juice to orange juice (*245*), making it possible to detect the addition of 5% lemon juice.

Techniques for determination of *Salmonella* in dried eggs have been reported (*12*), and a modified DU spectrophotometer is used at wave lengths of near 575 mμ for detecting blood in white shell eggs (*27*).

R_f values on filter paper chromatograms on a number of coumarins and related cinnamic acids in various solvents have been determined (*283*). A chromatographic method, using a specially prepared aluminum oxide, has been suggested for the separation of vanillin from coumarin (*210*), and ethylvanillin in vanilla extract is estimated by using a freshly prepared 1% *p*-aminophenol solution, which, in an alkaline medium, gives a dark purplish color in the presence of ethylvanillin (*140*).

A fermentation test is suggested for detecting preservatives other than benzoic or sulfurous acid in beverages (*205*) and in meat products (*204*). The diazotization method of Edwards has been used to detect benzoic acid and its derivatives in foods (*93*).

Chicory in amounts of 1% or more can be detected in coffee by the Seliwanoff test for fructose (*200*), and detection of adulteration in vinegar is based on its albuminoid ammonia value (*199*).

The proposal to employ dimethyl dichlorosuccinate as an antimycotic in packaging such foods as cheese, bread, fresh raspberries, and tomatoes created need for a method for its detection and determination. A colorimetric method sensitive to 1 p.p.m., based on the determination of degradation products of dimethyl dichlorosuccinate has been offered (*235*).

A colorimetric method, based on the action of the curd of the milk sample, with certain solvents, is used for detecting reconstituted milk in normal milk (*238*). Cow's milk added to goat milk is detected by a serological test (*272*). It has been reported (*313*) that use of tetrazole salts instead of methylene blue in testing the sanitary condition of fluid milk has no advantages.

Detection of adulteration of meat with horse meat depends upon the fact that horse fat contains a higher percentage of linolenic acid than does the depot fat of normal beef animals, sheep, or pigs (*54*); serological methods are also recommended for horse meat detection as well as the addition of pork to hamburger (*217*).

MISCELLANEOUS

Further work has been carried out on determination of ethylene in air (*279*).

Methods for sampling Cheddar cheese for commercial analysis have been described (*234*). Various methods of measuring the consistency of tomato puree have been evaluated (*60*), and a method for sampling tomatoes for grading has been reported (*160*). The Tenderometer for measuring texture of cooked frozen peas has been correlated with organoleptic evaluations of texture (*186*), and it has been suggested that maturity in frozen peas can best be measured by specific gravity and alcohol-insoluble solids (*166*).

Terpenes in citrus oils have been determined using filter paper impregnated with silicic acid (*213*) or glass plates coated with silicic acid (*239*); R_f values of many terpenes and oxygenated terpenes found in essential oils have been reported (*196*), and methods of analyses of lemon oil have been the subject of a review (*249, 305*).

An apparatus and procedure for separating and estimating volatile constituents in fruit products have been reported (*102*), giving fairly reproducible results on a variety of fruits.

A paper chromatographic method is suggested for determining caffeine and trigonelline in coffee (*157*); chemical procedures for estimating caffeine in coffee (*114, 242*) and in soft drinks (*133*), and theobromine in cocoa residues (*95*) have been proposed.

A statistical approach to measurement of particle size of dried milk has been presented (*141*), along with a semiautomatic cryoscope for routine determination of the freezing point of milk (*265*), a method for measuring the wettability of powdered milk (*11*), and suggested techniques for estimating total solids in milk (*50, 120, 167*).

One of the newer methods for enumerating bacteria is the molecular or millipore filter technique, which removes bacteria from aqueous solutions and permits growth directly on the filter itself (*37*).

Improved techniques for thermal process evaluation (*223, 227, 276, 282*) will be found helpful for those engaged in this field.

A new apparatus, made from ordinary laboratory equipment, makes possible a rapid method for determining alcohol in raisin residues (*33*). Benzoyl peroxide in flour and bread is determined absorptiometrically by Mohler's reaction (*153*), and testing of antifoam agents involves registering the foam within an enclosed standpipe through which air is bubbled at a determined rate (*82*). With comparatively simple apparatus, apparent density of meats can be approximated so that the data may be used to calculate piece weights in packing plants (*88*). Of interest, too, is a dilatometric method for control of fat hydrogenation (*91*) useful in hydrogenation plants.

Quality of active dry yeast is compared by using the methylene blue staining procedure, plate counts, and baking tests (*225*).

High voltage gaseous discharge phenomena have been employed for measuring the vacuum levels in commercial glass containers (*76*). The method is especially adaptable to measurement of relatively rapid gas leakage rates. An apparatus for sampling and analyzing swell gases from cans is also of interest (*274*). A suggested procedure for measuring gas permeability of sheet material is based on the principle of measuring a change in volume of constant pressure and temperature conditions (*32*).

Infrared absorption spectra of several water-soluble gums have been presented (*207*), useful for the identification of gums isolated from foods.

A rapid method for determining the solubility of dried whole egg powder involves dispersion of the sample in 0.9% saline, centrifugation of saline-insoluble solids, and precipitation of the saline-soluble and colloidal fraction with Esbach reagent, followed by centrifugation of the precipitate and reading the volume of the precipitate as the solubility index (*19*).

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Fertilizers

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THIS review covers the literature on analysis of fertilizers since the review published in the January 1953 issue of Analytical Chemistry and up to August 15, 1954 (*15*). It has been restricted to procedures that are recommended for fertilizer analysis or that appear to be adaptable for fertilizer analysis with little modification.

SAMPLING AND SAMPLE PREPARATION

Randle (*114*, *115*) presented an extensive table of analyses of 29 grades of fertilizer by 10 collaborators who used a hand-quartering procedure and of 22 samples by seven collaborators who used a riffle-quartering procedure. He also tabulated the results of analysis of fertilizers of 47 grades by 13 collaborators prepared by the official AOAC sample procedure. Fudge (*41*) reviewed current practices in collecting, storing, and sampling of nitrogen solutions and liquid fertilizers containing free ammonia.

NITROGEN

Reports on determination of nitrogen in fertilizers by numerous collaborators by AOAC methods, the Shuey method, and various modifications of the Kjeldahl method were made by Davis (*26*, *27*). Nakamura and Yamazoe (*96*) compared various methods for determining nitrate nitrogen in fertilizers; they concluded that the reduced iron method with steam distillation was best.

Numerous studies were made of the Kjeldahl method. McKenzie and Wallace (*81*) made a critical study of temperature, catalyst, and oxidizing agent in the digestion step. Lepper (*79*) studied nitrogen loss when a selenium mixture was used in the digestion step; he concluded that nitrogen loss is not serious and there is little danger of selenium poisoning. Rosicky (*119*) found that addition of kerosine, fuel oil, or mineral oil to the digestion flask was helpful in prevention of foaming. Dickinson (*28*) applied the Kjeldahl method to determination of nitrogen in nitrates. Perrin (*104*) developed a rapid modification of the Kjeldahl procedure which compared favorably to other procedures.

Hartmann and Bäthge (*59*) used dicyclohexylthallic ion as a precipitating agent for the gravimetric and volumetric determination of nitrates. Feigl and Schaeffer (*34*) treated alkali nitrates with concentrated formic acid and titrated the resulting carbonate; the procedure was also applicable for alkali nitrates.

Komarmy, Broach, and Testerman (*75*) developed a photometric procedure for high nitrate-containing solids in which phenoldisulfonic acid was used. Masek (*84*) described a polarographic method for nitrates and nitrites, in which uranium salts were found superior to lanthanum salts.

The analysis of gas mixtures containing oxides of nitrogen was studied by Johnson (*69*).

Yuen and Pollard (*150*) described preparation of the Nessler reagent for determining nitrogen in agricultural materials. Morgan and Kralovec (*89*) determined available fertilizer nitrogen in urea-formaldehyde compositions based on the nitrogen insoluble in cold water and that insoluble in a hot buffered phosphate solution. Rangier and Krebs (*116*) determined ammonia in the presence of large amounts of urea by a cold precipitation as the iodo-mercury complex. Rudorff and Zannier (*121*) described a titrimetric determination of potassium and ammonium in the presence of one another. Nagai and Kanazawa (*93*) determined nitrogen in a mixture of calcium cyanamide and fused phosphate fertilizer. Kondo (*76*) analyzed reagent grade ammonium sulfate by four different procedures.

PHOSPHORUS

Determination of available phosphoric acid in calcium metaphosphate was studied by Jacob, Caro, and Magness (*66*), who concluded that grinding of the sample to −60 or −80 mesh was necessary, depending on whether continuous or intermittent agitation was to be used during the citrate digestion. Jacob, Hoffman, and Schramm (*67*, *68*) compared the direct determination of available phosphoric acid in a number of fertilizers by volumetric and photometric procedures. Allen (*1*) described a direct determination of available phosphoric acid in fertilizers for screening purposes.

The gravimetric procedure for phosphate received the attention of several workers. Pribil and Jelinkova (*109*) precipitated phosphate as magnesium ammonium phosphate. Shibata and Tsukiyama (*128*) found 0.02% nitric acid better than 1% potassium nitrate with or without a small amount of nitric acid for washing ammonium phosphomolybdate precipitate. Cole and Wilson (*23*) used zirconium nitrate as a precipitant for phosphate. Hardin (*58*) described the use of a perchloric-nitric acid digestion.

Several volumetric methods were described. Rancke-Madsen and Kjaergard (*113*) neutralized the phosphate solution to the $H_2PO_4^-$ end point, then added cerium nitrate to liberate the remaining hydrogen ions, which were titrated with sodium hydroxide. Kindt, Balis, and Liebhafsky (*73*) titrated a phosphate solution with alkali after separation of calcium by ion exchange. Goudie and Rieman (*55*) found that an error was introduced in ion exchange procedures by the presence of carbonate and incomplete removal of ferric ion. Brouty and Chatelet (*17*) studied titration of phosphoric acid with lime and described the practical conditions necessary for accurate determinations.

There were numerous reports of colorimetric determination of phosphorus. Most methods depend on the formation of phosphovanadomolybdate complex. Gericke and Kurmies (*50*) described a procedure which was not found by Scheffer and Pajenkamp (*124*) to be in agreement with the usual gravimetric procedures. Teague (*138*) determined available phosphoric acid in fertilizers by combining the water and citrate extracts, followed by a photometric step. Bridger, Boylan, and Markey (*16*) described a colorimetric procedure for total and available phosphoric acid in several different fertilizers. Gee and Deitz (*49*) described a colorimetric procedure said to give results to within 1 part per thousand.

Other reagents were also used to develop color in photometric methods. Furukawa, Oida, Nakamura, Kasuga and Yoshikawa (*44*) compared several reagents. Takahashi (*136*) developed a color with metol reagent (prepared from p-MeHNC$_6$H$_4$OH and sodium bisulfite) and ammonium molybdate. Ikeda (*64*) used sodium sulfite-bisulfite, sodium thiosulfate, ammonium molybdate, and sodium acetate. Furukawa, Nozue, and Yoshikawa (*43*) used sodium molybdate and N-methyl-p-aminophenol in sodium bisulfite, and Kato and Ooizumi (*71*) used ammonium molybdate either with or without pyrogallol.

Other instrumental methods were also used by a number of workers. Austin, Denson, and Epps (*5*) found that a photometric method for available phosphorus pentoxide was comparable in accuracy to the official AOAC method. Dippel, Bricker, and Furman (*29*) used a flame photometric procedure. Nakamura and Kishimoto (*95*) and Novak (*98*) used a method in which excess molydenum was determined by a polarograph.

Nagai, Kanazawa, and Ishii (*94*) described a method of determining citric acid solubility of a mixture of calcium cyanamide and fused phosphate fertilizer. Krumm (*77*) defined limitations of the citric acid solubility method when applied to phosphate rock. He concluded that this method bears no relation to the actual availibility of phosphate rocks in field practice.

Shapiro (*127*) described a simple field method for determination of phosphate in phosphate rock, which was accurate to within 5% of the phosphate content.

Etienne (*33*) separated meta-, pyro-, poly-, and orthophosphates by progressive precipitation of their barium salts.

POTASSIUM

The results of analysis of several fertilizers by numerous collaborators who used the AOAC method, the Perrin method (*103*), and the flame photometric method were compared by Ford (*38–40*); the results were in good agreement. The presence of minor elements (copper, manganese, cobalt, and others) resulted in variations in the results by the Perrin and Lindo-Gladding methods (*4*) but not by the flame photometric method.

Gravimetric procedures were reported by a number of workers. Eser (*32*) reported that a large error in the Cramer and Tisdall method (*24*) is due to the high solubility of potassium sodium cobaltinitrite; this can be avoided by using a saturated solution of the precipitate for washing. Chiba (*20*) reported that an error in the cobaltinitrite method is caused by the presence of ammonium salts in the reagent. The method based on precipitation of potassium as the 12-phosphomolybdate was described by Belcher and Robinson (*8*). Berkhout (*9*) described a modification of the method of Raff and Brotz (*112*) which precipitates potassium as a salt of tetraphenylboron. Procedures were described by Flaschka, Amin, and Holasek (*36*) and Rudorff and Zannier (*120, 121*), who titrated potassium tetraphenylborate iodometrically. A volumetric method depending on iodometric titration of potassium diliturate with copper sulfate was studied by Press and Murray (*106*). Ievin'sh and Ozol (*63*) precipitated potassium with a reagent consisting of tartaric acid, pyridine, and alcohol followed by titration with sodium hydroxide.

Numerous workers studied the flame photometric method. Mehlich and Monroe (*86*) investigated statistically reasons for large variations in results of a collaborative study. Schall and Hagelberg (*122*) analyzed representative grades of fertilizers made from reagent grade ingredients; they concluded that the results were comparable in accuracy to those by the AOAC method. Crooks (*25*) described a rapid flame photometric method suitable for screening mixed fertilizers. Eggertsen, Wyld, and Lykken (*31*) found that interference caused by the presence of acids and salts in the test solution can be eliminated by using a buffer such as lithium chloride. Blackwell, Yeager, and Kraus (*13*) analyzed 28 samples of fertilizers and found the results as accurate as those by the AOAC procedure.

Various other methods for potassium included an indirect polarographic method of Monnier and Besso (*88*). Conductometric methods were used by Udovenko and Pasovskaya (*141*), who titrated a sodium picrate solution, Weiner and Koller (*143*), who titrated a precipitate of potassium chlorate, and Yasumori (*148*), who titrated with sodium dipicrylaminate. Radioactive measurements were used by Scheel (*123*) and Zlotowski (*152*). A turbidimetric method was used by Olson (*99*).

CALCIUM

Work on gravimetric procedures of calcium determination included a study by Matsuo (*85*), who prevented precipitation of magnesium in the oxalate method by careful control of precipitation conditions. Gautier and Renault (*48*) found an error in the determination of calcium as sulfate because of inclusion of calcium oxide or carbonate; this was prevented by treating the oxalate with nitric acid before addition of sulfuric acid. Graue and Zohler (*56*) gave detailed directions for determination of calcium and magnesium in ores and slags with uranildiacetic acid; interfering heavy metals were removed by precipitation with ammonium sulfide in the presence of ammonium chloride.

Shinkai (*129*) determined calcium and magnesium in dolomite by using barium hydroxide instead of calcium hydroxide to precipitate magnesium hydroxide; they also described a method in which calcium oxalate was titrated with potassium permanganate.

Several workers studied volumetric methods in which chelating agents were used. Berkhout and Goosens (*10*) determined calcium and magnesium in fertilizers by precipitation of calcium oxalate in the presence of acetic acid and titration of the filtrate with Complexon [(ethylenedinitrilo)tetraacetic acid]. Banks (*6*) confirmed that the ethylenediamine tetracetate method for calcium and magnesium is rapid and reasonably accurate. Pribil and Fiala (*108*) precipitated calcium oxalate in the presence of disodium ethylenediamine tetraacetate and determined the calcium as sulfate by manganometric titration or Complexon titration. Calcium, magnesium, and iron in limestone were determined by Cheng, Kurtz, and Bray (*19*) by digestion in perchloric acid and titration with disodium (ethylenedinitrilo) tetraacetic acid. Jordan and Robinson (*70*) determined calcium and magnesium in limestones by titration with disodium ethylenediamine tetraacetate. Brunisholz, Genton, and Plattner (*18*) determined calcium in the presence of magnesium and phosphate ions by passing the solution through an ion exchange resin and titrating with disodium ethylenediamine tetraacetic acid. Stephens (*134*) determined calcium and magnesium by titration with Complexon.

Calcium in magnesite was determined by Flaschka and Huditz (*37*) by precipitation with N-hydroxynaphthalimide. Goto and Kakita (*54*) determined free lime in slag by digestion with ethylene glycol and titration with hydrochloric acid. Kurmies

(*78*) determined calcium in Thomas phosphate by precipitation in the presence of ammonium acetate and titration with potassium permanganate.

Several instrumental methods for calcium were described. Williams and Moser (*146*) used ammonium purpurate in a colorimetric method. Ashizawa (*2, 3*) used anthranilic acid in a colorimetric method. Pribil and Vicenová (*111*) used a polarographic method based on the interchange between the complexonate of zinc and calcium. Musha (*91*) used a high frequency titration with ammonium oxalate. Gjems and Lydersen (*53*) found that the reduction in calcium flame intensity due to aluminum in the flame photometric method was eliminated by precipitation of the aluminum with benzoate. Ikegami and Matsuo (*65*) studied the effect of manganese in the determination of calcium in basic slags.

MAGNESIUM

Smith and Olney (*131–133*) reported results of collaborative studies on determination of magnesium by AOAC procedures and chelate procedures; they also studied the effect of particle size of the fertilizers.

A review of gravimetric procedures for determination of small quantities of magnesium was published by Bourdon (*14*). A titrimetric procedure for magnesium in dolomite was described by Remy-Gennete (*118*).

Tanimoto and Morita (*137*) described a colorimetric procedure with Titan Yellow. Zan'ko and Panteleeva (*151*) used an amperometric procedure with 8-quinolinol (oxine). Pasovskaya and Udovenko (*102*) described four conductometric methods: titration of an aqueous alcoholic solution of the sulfates by barium hydroxide and barium acetate; titration of this solution by barium hydroxide, (*p*-nitrobenzeneazo)-1-naphthol, and barium acetate; titration of this solution by barium hydroxide; and a similar method, except that a graphical technique was used.

Several who studied calcium also described procedures for magnesium (*6, 10, 19, 56, 70, 129, 134*).

SULFUR

Sulfates were determined in the presence of iron by Gandolfo (*47*) by treatment of the solution with hydrochloric acid and potassium iodide, addition of sodium hydroxide, and precipitation of barium sulfate. Sulfur in blast-furnace and open-hearth slags was determined by Morimoto (*90*) by a combustion method in which iron powder or boric oxide was added to the sample. Sulfur in ammonia synthesis gases was estimated by Thampy (*139*) by passing them through alkaline potassium permanganate solution and an aqueous alcoholic solution of potassium hydroxide and hydrogen peroxide.

BORON

Berry (*11, 12*) reported the results of several collaborators who analyzed three samples of mixed fertilizers by two AOAC methods, the Virginia modified method and a spectrometric method. The less soluble borates in fertilizers were determined by Naftel and Fajans (*92*). Boron in superphosphate, Rhenania phosphate, and Rochling phosphate was determined by three different methods by Wehrmann (*142*). Polheim (*105*) determined borox in phosphates by precipitating the phosphate with ferric chloride and calcium carbonate before titrating the boron. Schutz (*125*) described an ion chromatography method. Singh (*130*) used a spectrochemical method. Mitrovic (*87*) determined the lower limits of sensitivity in spectrochemical determination of boron. Otting (*101*) published a comprehensive treatise covering a critical investigation of the various methods of determining boric acid in plant and animal products.

MANGANESE

Luiggi (*80*) used hydroxylamine, hydrazine, and semicarbazide to reduce manganese to the divalent state to separate it from iron and rare earths before proceeding with the analysis.

Gibaud (*51*) used an iodometric method in which a manganese complex with triethanolamine was reduced by potassium iodide and titrated. Neelakantam and Parthasarathi (*97*) used a volumetric method in which manganese oxinate was precipitated, dissolved in hydrochloric acid, and titrated with potassium bromate-bromide solution with indigo carmine as an indicator. Smith (*131*) reported collaborative analyses of manganese in fertilizers.

COPPER

Halbrook (*57*) determined copper and zinc in fertilizers by a polarographic method which gave results comparable with those obtained by the AOAC method. Divis and Skoda (*30*) used a colorimetric method with the methyl ether of 2-isatoxime.

ZINC

Chelating agents were used by Flaschka (*35*), Kinnunon and Wennerstrand (*74*), and Strafford (*135*). Honjo (*61*) described a volumetric determination in which zinc was precipitated as sulfide, dissolved in hydrochloric acid, and titrated with sodium hydroxide. Kato and Takei (*72*) used a dithizone colorimetric method in which interfering ions were masked by bis(hydroxyethyl) dithiocarbamate. Udovenko and Pasovskaya (*140*) used a conductometric titration with barium hydroxide. Halbrook (*57*) determined zinc in fertilizers polarographically.

COBALT

A spectrophotometric determination of cobalt with 2-nitroso-1-naphthol-4-sulfonic acid was described by Wise and Brandt (*147*). Gagnon (*45*) used a colorimetric method depending on the pink color of cobalt sulfate. Gallego, Deijs, and Feldmeijer (*46*) determined traces of cobalt in fertilizers by a colorimetric method. Pribil, Jenik, and Kobrova (*110*) described a colorimetric method in which sodium diethyl dithiocarbamate was used.

MOLYBDENUM

A gravimetric procedure for molybdenum was described by Ray (*117*), in which molybdenum sulfide was precipitated and ignited to molybdenum trioxide. In a photometric method for traces of molybdenum described by Henrickson and Sandell (*60*), molybdenum was precipitated as the sulfide and determined by the thiocyanate–stannous chloride method. Colorimetric methods were described by Will and Yoe (*144, 145*), in which molybdenum was determined by reaction with disodium-1,2-dihydroxybenzene-3,5-disulfonate (Tiron) and with mercaptoacetic acid. Polarographic methods were described by Yokosuga (*149*) and Pribil and Blazek (*107*).

FLUORINE

The determination of fluorine in compounds containing halogens, sulfur, phosphorus, and arsenic by the lead chlorofluoride method was described by Belcher, Caldas, and Clark (*7*). Gillis, Hout, and Kemp (*52*) determined fluorine in slags by a spectrochemical method. MacNulty, Reynolds, and Terry (*82, 83*) used a polarographic method. Funasaka, Kawane, Tabuchi, and Kojima (*42*) presented a critical discussion with a statistical background of various methods for determination of fluoride.

BASICITY

Clark and Gaddy (*22*) reported a collaborative study of the carbonate carbon or calcium equivalent and acid-insoluble ash.

content of several fertilizers. Horton and Newsom (*62*) described a rapid gas evolution method for calcium carbonate equivalent in liming materials.

GENERAL

The Organisation of European Economic Cooperation described methods of analyzing fertilizers used in OEEC countries (*100*).

Chichilo and Whittaker (*21*) determined the major constituents and trace element content of a number of blast-furnace, basic, and phosphorus-furnace slags. Servigne (*126*) also reported analytical results on a large number of phosphatic slags.

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Pharmaceuticals and Natural Drugs

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THREE years have elapsed since publication of the latest in this series of reviews of analytical methods used in the analysis of pharmaceuticals and natural drugs (*245*). The intervening time has been filled with an annually increasing number of articles pertaining to this field. The present review covers the period to 1954. Much of the changing emphasis in methodology will be reflected in the 15th revision of the United States Pharmacopoeia and in the tenth revision of the National Formulary; both of these compendia are scheduled for publication in 1955. Both will incorporate many more of the newer instrumental techniques among the official methods of analysis.

As in the past, considerable selectivity has been exercised in reporting methods of assay for various products. The reasons for this selection remain strictly a matter of opinion on the part of the authors; in many cases, laboratory experience with the methods described has provided a background for more critical discussion of the principles and techniques involved; in other cases, an opportunity to use the methods described has not presented itself and comment is necessarily limited to theoretical considerations.

It is obvious that all the many thousands of pertinent papers which have appeared in the literature since 1951 cannot be mentioned in this or any other review which endeavors to remain current in its coverage and hopes to retain sufficient continuity to be—albeit laboriously—readable. The authors again wish to insist that the exclusion of published articles from this review is not an indication of a lack of value of the methods described. It is unquestionably true that in this as well as in other scientific domains, there is much in the way of published information which is unnecessary and of doubtful value to other workers. Wherever possible, these reports of highly restricted applications or of minor modifications made to fit a very specific set of circumstances have been avoided.

The writers feel that the previous classifications of chemical, physiochemical, and physical methods are no longer of particular significance, since most pharmaceutical laboratories are now equipped to utilize instrumental methods of analysis; consequently, the present review is concerned with classification solely along the lines of the types of substances to be assayed. The substance classification has been extended somewhat to facilitate locating methods for specific compounds.

ALKALOIDS

Analytical methods for the analysis of alkaloidal constituents, both in crude drugs and in pharmaceutical combinations, are one of the most important groups in the field of pharmaceutical analysis. Concentration levels in raw materials are generally low, as are the amounts which are therapeutically active; thus, procedures for assay are generally devised for the micro or semimicro range. The isolation of alkaloids from natural sources usually involves separation from closely related substances which may have markedly different activities. One is concerned, then, with as great specificity as possible as well as with separation procedures which will yield relatively uncontaminated products for measurement or identification.

Partition chromatography involving incorporation of the sample into a stationary phase in a Celite support has been used by Banes (*16*) for the separation of several alkaloids including strychnine, quinine, and caffeine. New continuous extraction methods for the isolation of organic bases such as ephedrine, codeine, and papaverine have been reported; extractors for use with aqueous solutions and organic solvents both lighter than and heavier than water are described in detail (*324*). Alkaloids have been titrated as bases in an acetic acid medium with perchloric acid (*138*). An interesting general method for the determination of alkaloids involves an application of Valser's reagent. Under proper conditions, the insoluble iodomercuriates of organic bases form suspensions, the apparent absorption of which can be related to concentration. A blank turbidimetric reading is obtained by addition of excess potassium iodide which dissolves the suspension (*311*). Data sufficient for the identification and differentiation of *N*-allylnormorphine and morphine are available from the infrared mull spectra. Quantitative evaluation of *N*-allylnormorphine is possible by ultraviolet absorption measurements at 285 mμ or by polarography (half-wave potential, − 1.6 volts *vs.* S.C.E. in 2*M* lithium chloride) (*333*). After preliminary extraction of atropine sulfate or scopolamine hydrobromide, carbon tetrachloride solutions have been used for quantitative infrared analysis of these compounds in ointment preparations (*395*).

A relatively specific spectrophotometric method for the determination of atropine in crude belladonna drug is reported by Worrell and Booth (*412*). In this procedure, atropine base reacts with copper sulfate and naphthenic acid in benzene to give a stable complex with an absorption maximum near 700 mμ. It has been observed that caffeine precipitates quantitatively from aqueous solution with phosphomolybdic acid. The precipitate is soluble in acetone, and its color can be measured in this solution at 440 mμ. The method has been applied to APC mixtures; there are some interferences from other antipyretics and alkaloids (*91*). The murexide test for caffeine and theobromine has been applied to a very large number of compounds; the characteristic color given by caffeine and theobromine was duplicated only by theophylline and uric acid. A pink color was reported for xanthine, alloxanthine, and a few other compounds. Very few interferences were noted (*100*). Caffeine has been separated from aspirin and phenacetin by partition chromatography, using water as stationary phase in a silicic acid support. The eluting phase for caffeine is chloroform (*181*).

Chromatographic methods have been applied to the determination of codeine in opium and other complex mixtures; fractionation occurs on elution from an alumina column with isopropyl alcohol–chloroform-benzene. The eluate fractions are titrated and ultraviolet absorption ratios used to establish a potency of various fractions. Morphine is extracted from the original benzene solution with saturated sodium acetate solution. It would appear that ultraviolet absorption measurements could also be used for quantitative evaluation of the fractions in place of the titration procedure described (*237*). Countercurrent distribution data for the separation of colchicine, isocolchicine, and colchiceine ethyl ether have been reported for the system ethyl acetate–water (*11*). A colorimetric method for the assay of ephedrine requires reaction of the alkaloid in benzene solution with picryl chloride. The color developed when the reaction mixture is heated is measured at 400 mμ. The reaction is not complete, requiring carefully controlled conditions (*77*). A modification of the conventional fluorescence reaction of epinephrine and norepinephrine with ethylenediamine permits the differentiation of these two substances. The addition of sodium hydrosulfite just before condensation with ethylenediamine reduces the fluorescence of norepinephrine to 22% of its original value without affecting the fluorescence of epinephrine (*242*). A semiquantitative test for norepinephrine in epinephrine utilizes paper chromatographic separation in an ascending butanol–acetic acid–water system. The component zones are developed with potassium iodate or potassium ferricyanide solution and the zones compared visually with standards (*402*).

A great many methods for the separation of the alkaloids of ergot have appeared in the recent literature. Both qualitative and quantitative procedures have been devised. Buffered filter papers employing an organic mobile phase have been satisfactorily used (*26, 28, 379*). Other techniques involve the use of formamide to impregnate paper (*236, 287*). A quantitative procedure for determination of ergot alkaloids on paper chromatograms has also been described. In this method the fluorescent spots are detected by ultraviolet light and cut from the strip. The cutout portions are extracted with dilute tartaric acid solution and color is developed with *p*-dimethylaminobenzaldehyde. After filtration, the absorbance of the solution is measured. The author claims substantially 100% recovery from experimental mixtures of alkaloids and claims that the method can be employed for the quantitative separation of most of the ergot alkaloids including ergocryptine, ergocornine, and ergocristine (*286*). A new color reaction for ergot alkaloids which does not involve the indole group is obtained by spraying paper chromatograms with dilute solution of ninhydrin in butanol and heating. The reaction is also observed with lysergic acid (*27*). In a study of several extractants for ergot alkaloids, Campo and Gramling (*74*) consider the azeotrope of chloroform and tetrahydrofuran to be the best solvent for crude drug extraction.

A fluorometric method for evaluation of hydrastine in hydrastis involves oxidation of the alkaloid to hydrastinine (fluorescent) with nitric acid; canadine and berberine do not interfere (*53*). Certain of the tropane alkaloids, including hyoscine and hyoscyamine, have been separated on phosphate-buffered paper; the mobile phase was water-saturated 1-butanol. After the zones of alkaloids have been located on a guide strip with Dragendorf's reagent, sections are cut out, eluted, and estimated with a modified Vitali-Morin reaction. Reported recoveries were of the order of 65 to 75%; a correction factor was used for quantitative evaluation (*99*). The tropane alkaloids have also been separated using partition chromatography and quantitatively estimated by titration of eluted fractions with standard acid (*111*).

Morphine has been separated from codeine by means of exchange on a strong anion resin (*151*). These two alkaloids may also be distinguished by observing the characteristic shift of the ultraviolet absorption maximum of morphine to longer wave lengths in alkaline solution. A two-component ultraviolet analytical method is suggested (*81*). The iodic acid–ammonium carbonate–nickel salt reaction has been adapted for the routine spectrophotometric assay of morphine. The method has been used for the rapid determination of morphine in opium; only pseudomorphine interferes weakly among coexistent bases. Variables in the method were carefully studied (*296*). The characterization of the complex formed by reaction of morphine with potassium mercuric iodide has resulted from the work of Levi and Farmilo (*228*). The ultraviolet and infrared spectrum, specific rotation, x-ray pattern, and solubility behavior of the morphine complex are reported for forensic purposes. The isolation and purification of semimicro quantities of morphine involve modification of conventional extraction procedures to provide maximum yield. Ion exchange procedures were employed (*2*). A combination extractor-adsorption column has been used in the isolation of morphine in opium. The adsorbent is Florisil, which removes impurities (*209*).

Some improvements in the colorimetric assay for pilocarpine and its salts have been described by Webb *et al.* (*399*). In this procedure the formation of the unstable free alkaloid is avoided. Pilocarpine has been assayed in ointment preparations in combination with phenacaine by an extraction process followed by infrared absorption measurements in chloroform solution (*396*). Quinidine sulfate and strychnine nitrate have been assayed by reaction in aqueous solution with potassium mercuritetrathiocyanate; after removal of the precipitate of the alkaloid with this substance, the excess reagent solution may be titrated by addition of excess silver ion, removal of the precipitate, and titration of the remaining silver with thiocyanate (*212*). Quinine and strychnine have been differentiated quantitatively in a simple two-component analytical scheme involving differences in the ultraviolet absorption spectra of these compounds (*34*). The behavior of quinine on several types of alumina columns has been studied (*43*). It was observed that basic aluminum hydroxides adsorb the alkaloid from aqueous solutions; it may be later eluted with water as the free base.

Ultraviolet absorption measurements permit the determination of strychnine in the presence of brucine (*33*). These two compounds have been separated also by chromatography on alumina from other interfering substances; the alkaloids are not retained on the column in 86% ethanol. Brucine is oxidized with potassium persulfate and the ultraviolet absorption of the remaining strychnine is measured at 254 mμ (*108*). Theobromine in 1 to 1 acetic acid–carbon tetrachloride has been titrated with perchloric acid in glacial acetic acid. The indicator (α-naphtholbenzein) change is yellow to green (*292*). The procedure evades possible interference from acidic tablet excipients. Theobromine may be differentiated from caffeine by ultraviolet spectrophotometry in alkaline solution. Acidic solutions of both alkaloids have similar maxima, but theobromine shows a considerable increase in absorption in the 220 to 254 mμ range in alkaline solution (*253*). An ultraviolet spectrophotometric method for the determination of theophylline in pharmaceuticals has been compared with the silver salt procedure (*85*). The instrumental method compares well with the official procedure and avoids some errors.

The recent enhanced interest in reserpine and related alkaloids and also the veratrum alkaloids has not as yet produced much information concerning specific separation and measurement procedures for these substances. Some techniques for total alkaloid estimation in *R. serpentina* have involved extraction methods in a more or less conventional pattern (*186, 187*). A colorimetric assay for yohimbine involves reaction with *p*-dimethylaminobenzaldehyde and hydrogen peroxide (*213*). Infrared spectra of several veratrum alkaloids have been reported (*271*), and the intensity of the infrared band due to carbonyl absorption from the ester group was found to parallel the hypotensive activity in drugs. It cannot be expected, however, that the respective hypotensive activity of pure ester alkaloids is simply a function of their degree of esterification. A paper chromatographic procedure for separation of certain of the veratrum alkaloids has been reported recently (*152*). This same report discusses titrimetric methods, infrared absorption procedures, nonaqueous titration, and ultraviolet absorption of the alkaloids; it describes in detail a quantitative paper chromatographic method for the estimation of protoveratrine A and protoveratrine B.

ANTIBIOTICS

Although the ultimate appraisal of antibiotic potency may only be made in terms of in vitro or in vivo action against a particular organism, chemical methods of relatively high specificity have been extremely useful as control procedures. A fluorometric assay of aureomycin utilizing concentrations as low as 5 γ per ml. has been described (*320*). Separation of the antibiotic is accomplished by means of butanol extraction or adsorption on fuller's earth. Several antibiotic salts including those of Aureomycin, erythromycin, Magnamycin, and Terramycin have been titrated in nonaqueous media. The halide salts require preliminary addition of mercuric acetate before titration of the salt with perchloric acid in dioxane (*337*). Aureomycin and Terramycin have been identified on paper chromatograms by spraying with a dilute solution of *p*-dimethylaminobenzaldehyde under the conditions described. Aureomycin produces a dirty yellow spot and Terramycin gives a blue-green color. Five micrograms of the antibiotics per square centimeter may be detected (*349*). A sensitive color reaction for chloramphenicol involves solution of the antibiotic in purified pyridine, addition of potassium hydroxide, and heating with agitation for 5 minutes at 100° C. On cooling, a rose to red color develops in the pyridine layer. Sensitivity is 10 γ of the antibiotic (*378*). Higuchi and coworkers (*180*) report the partition chromatography of chloramphenicol. The stationary phase is water in a silicic acid support, while the eluting solvent is 10% ethyl acetate in chloroform; the eluate fractions are examined spectrophotometrically at 278 mμ. Acid hydrolyzates of Aureomycin, Carbomycin, erythromycin, and Terramycin all react with arsenomolybate to produce blue complexes; only Terramycin will reduce the reagent prior to hydrolysis. Colorimetric assays are devised covering the range 2 to 160 γ, depending on the antibiotic (*277*).

The reaction of the erythromycin in the presence of strong sulfuric acid to produce an intense yellow color has been made the basis for a colorimetric procedure. Degradation products of erythromycin interfere. The procedure is reproducible to within about ±3% when applied to fermentation beers and about ±1% on aqueous solutions of purified materials (*122*). Quantitative infrared measurement of erythromycin involves determination of absorbancy in the 10.5-micron region (*397*). An ultraviolet absorption method involving preliminary treatment of erythromycin with alkali has been adapted to the determination of this antibiotic in various pharmaceutical preparations (*220*). A colorimetric method for the evaluation of Framycetin has been reported by Penau *et al.* (*276*). Several physical and chemical properties of Magnamycin, including melting point, specific rotation, and ultraviolet absorption spectrum, have been reported (*368*). The observation that ethanol solutions of micrococcin yield a purple fluorescence in ultraviolet light has provided a method of assay for this antibiotic (*173*). A method of assay for neomycin is based on the observation that neomycins B and C when heated in strong mineral acid yield furfural as one of the decomposition products. Quantitative evaluation is accomplished by measuring the absorbance of the solution at 280 mμ (*103*).

A rapid method of determining penicillin in culture fluids or in crude preparations involves preliminary extraction of penicillin from an aqueous phase with amyl acetate solution followed by re-extraction into a known volume of phosphate buffer at pH 7. The penicillin is then assayed by the iodometric titration method. A correction factor is introduced for substances in the culture fluid that are extracted and that take up iodine under these conditions (*25*). In the presence of small amounts of mercuric chloride at room temperature, penicilloic acid quantitatively reduces arsenomolybdic acid; when penicilloic acid is heated in dilute mineral acid in a boiling water bath, however, the reducing power is lost. These observations have been made the basis for the determination of this substance and of penicillin in fermentation samples (*268*). Pan and Perlman (*269*) have modified the Kapeller-Adler method for nitration of phenylacetic acid to provide nitration at a lower temperature. This technique yields only one nitration product instead of the several products obtained previously as shown by paper chromatography. The method is useful in determining phenylacetic acid and phenylacetamides in samples from fermentations. Penicillin labeled with sulfur-35 has been prepared by fermentation in a medium containing $S^{35}O_4^{--}$ in yields up to 30% on the basis of labeled sulfate. An isotope dilution procedure utilizing the labeled compound thus produced is described (*150*). The procedure cannot compete with chemical and biological methods on a cost basis, but it more nearly approaches an absolute measure of the penicillin content; 95% confidence limits for a single sample in duplicate are ±5.9% of the mean.

A method for the estimation of penicillin G in penicillin O involves oxidation, extraction of the benzoic acid produced from other ultraviolet-absorbing impurities, and measurement at 224 mμ. Recovery is 86 ± 4% (*199*). It has been found that *N,N'*-dibenzylethylenediamine-penicillin may be dissolved directly in alkaline solution for assay and allowed to stand 15 minutes after making to volume, and an aliquot iodinated in the usual iodometric procedure for penicillin assay. A blank value is obtained by treating an aqueous suspension of the insoluble drug with iodine; on dilution, the total reaction volume becomes sufficient to dissolve the salt (*272*). The utilization of ethylene oxide in sterilizing bulk penicillin has required development of a method for determining ethylene glycol (from hydrolysis of ethylene oxide) in procaine penicillin. The procedure is based on the periodate oxidation of ethylene glycol to formaldehyde and colorimetric determination of the formaldehyde with chromotropic acid (*50*).

Dihydrostreptomycin yields 1.02 moles of formaldehyde per mole and streptomycin 0.20 mole per mole when treated with periodic acid. A method for dihydrostreptomycin is based on the evaluation of the formaldehyde content of an oxidized sample with chromotropic acid. A correction is made for streptomycin interference when present in excess of 2%; the procedure requires careful control of variables (*381*). Monastero (*256*) has reported the colorimetric determination of streptomycin and dihydrostreptomycin which involves a reaction of the streptidine moiety of the molecule; the color reaction is an extension of a test for guanidine. The Voges-Proskauer reaction has also been adapted to the quantitative determination of streptomycin and dihydrostreptomycin (*365*). The streptomycins, streptothricins, and neomycins have been separated by paper chromatography (*193*).

Methanolysis of mannosidostreptomycin or dihydromannosidostreptomycin yields methyl mannoside, streptidine, and methyl streptobiosaminide dimethyl acetal or its dihydro analog, respectively; the basic products may be removed by passage over a cation exchanger. The mannose present in the effluent as methyl mannoside may be converted to the dinitrophenylosazone, which forms a purple color in alkaline solution; the color intensity may be directly related to the mannosidostreptomycin or dihydromannosidostreptomycin content of the sample (*229*).

CHEMOTHERAPEUTIC AGENTS

Several sympathomimetic amine salts have been converted to the free bases by passage over carbonate-regenerated Amberlite IR-45 ion exchange columns. The bases may be washed out of the column with 75% ethanol and titrated (*387*). Paper chromatographic separation and identification of sympathomimetic amines have also been reported (*407*). The separation of *m*-aminophenol, present in trace amounts in *p*-aminosalicylic acid, has been accomplished by paper electrophoresis; a similar procedure affords the separation of trace amounts of *p*-aminobenzoic acid in novocaine (*391*). Trace amounts of *m*-aminophenol in the presence of *p*-aminobenzoic acid have also been determined polarographically after diazotization and reaction with potassium carbonate (*202*), and by a colorimetric procedure (*334*). *p*-Aminobenzoic acid and its sodium salt have been estimated by titration in glacial acetic acid using perchloric acid as titrant; in this titration it is necessary to add chloroform to the solution after about 90% neutralization to precipitate the reaction products and to sharpen the end point (*67*). Sodium *p*-aminosalicylate in a water–ethylene glycol–isopropyl alcohol mixture has been titrated with perchloric acid, potentiometrically (*359*). A comparative study of several methods for the determination of *p*-aminosalicylic acid has been made by Rao (*300*), who proposes a method based on both decarboxylation and diazotization. A rather specific color reaction for *p*-aminosalicylic acid is observed on heating with a reagent composed of 1 to 1 concentrated hydrochloric acid–formalin. Neither salicylic acid nor *p*-aminobenzoic acid interferes (*319*).

The ultraviolet absorption spectrum of phenylacetone, an intermediate in the synthesis of amphetamine, has been used to determine the amount of the intermediate present in the drug (*128*). A general method for the titration of antihistamine bases and their salts is described by Kleckner and Osol (*208*); methods discussed include titration with aqueous standard acid or dissolution in excess standard acid and back-titration with alkali, and titration with perchloric acid in dioxane of a solution in glacial acetic acid.

Ascaridole has been determined in oil of chenopodium and in castor oil solutions of chenopodium by a polarographic procedure; the electrolyte is lithium acetate–lithium chloride–acetic acid in aqueous alcohol (*22*). Comparative ultraviolet absorption spectra of acetylsalicylic acid and of salicylic acid have been reported and a method for evaluation of binary mixtures of the two compounds has been developed (*376*). The separation of caffeine in the presence of phenacetin and aspirin has been accomplished by precipitation of caffeine periodide, thus providing a chemical procedure for the complete evaluation of APC mixtures (*409*). Higuchi and Patel (*181*) have also separated the components of APC combinations by a partition chromatographic procedure. A method has been described for the quantitative determination of atrolactamide (2-phenyl-3-hydroxypropionamide). The procedure is based on the conversion of this drug to iodoform and measurement of the absorbancy of a chloroform extract containing the iodoform isolated from the sample (*336*). A new colorimetric procedure for the determination of Benadryl (β-dimethylaminoethylbenzhydryl ether hydrochloride) has been described by von Horn (*388*).

Direct titration of barbiturate or sulfonamide tablets has been accomplished by Fritz and Vespe, who dissolved the samples in dimethylformamide and employed sodium methoxide in benzene-methanol as titrant (*386*). Barbital, allobarbital, pentobarbital, and—by adjustment of pH with borate buffer—phenobarbital, may be precisely assayed argentimetrically (*78*). Ribley *et al.* have utilized the infrared spectra of closely related barbituric acid derivatives for differentiating these compounds in mixtures (*307*). Considerable information has appeared recently on more specific methods of identification of barbiturates based primarily on paper chromatographic separations. Among those systems recommended are 1-butanol–5*N* ammonia (*4*) and chloroform-buffer, with the paper impregnated with buffer solution (*413*). Methods of identification of barbiturates on paper chromatograms have been investigated by Grieg (*154*) and Wickström *et al.* (*406*). The estimation of several barbiturates in biological materials has involved differences observed in the ultraviolet absorption spectra of solutions at pH 10.5 and in stronger alkali. Quantities as small as 0.1 mg. per 100 ml. of blood or 0.3 mg. per 100 grams of tissue may be determined (*145*). The ultraviolet absorption spectrum of phenobarbital in pH 9.5 borate buffer has been utilized to assay this compound in official preparations (*248*). Stearate interference in phenobarbital tablets has been eliminated by precipitating barium stearate from alcoholic solution with saturated barium hydroxide solution (*270*).

A colorimetric procedure for the evaluation of certain anthracene derivatives has been utilized to evaluate *Cascara sagrada* and some of its preparations (*117*). A system for the paper chromatography of chlorophylls incorporates filter paper strips impregnated with sugars and run in an ascending solvent consisting of 0.5% *n*-propyl alcohol in *n*-hexane (*353*). A method for the estimation of Dormison (methylparafynol) in biological material involves formation of an insoluble silver derivative; the complex is collected and decomposed and the silver content determined colorimetrically (*278*); the procedure was subsequently modified to include an aeration train to transfer the active substance from the sample to an alkaline silver reagent (*279*). Some difficulties in the accumulation of carbonate in the alkaline silver reagent—extracted from the air used in the aeration step—could possibly have been avoided by sweeping the sample with nitrogen.

Gentisic acid has been assayed in serum by preliminary acetone-alcohol extraction, oxidation by ferric ion, and colorimetric determination of the resulting ferrous ion; excess ferric ion is complexed with fluoride (*232*). A spectrophotometric study of gentisic acid has included observation of the formation of a green color with molybdophosphotungstate solution as well as characteristic ultraviolet absorption maxima of gentisic acid itself (*267*). Numerous methods for the evaluation of isonicotinic acid hydrazide have been reported, probably out of proportion to the difficulties involved in its determination. Those procedures which appear to be most satisfactory include nonaqueous titration (*5*), reaction with iodine followed by back-titration of excess iodine (*71*), a similar bromimetric procedure which avoids lactose interference (*170*), and a colorimetric procedure based on the reduction of ferricyanide with subsequent formation of Turnbull's blue on heating (*194*). For qualitative and quantitative purposes, the infrared mull spectrum and the ultraviolet absorption spectrum of isonicotinic acid hydrazide have been reported as well as some information on the polarography of this substance (*261*).

The quantitative differentiation of mixtures of certain ethyl mercuric and phenyl mercuric derivatives has been accomplished by observing their relative stability in strong hydrochloric acid solution (*289*). A paper chromatographic system for the separation of octamethylpyrophosphoramide utilizes propylene glycol–impregnated paper with a mobile phase consisting of 1:1:1 α,α,α-trichlorotoluene–carbon tetrachloride–toluene (*243*). Phenolphthalein-chocolate preparations have been estimated by nonaqueous titration; the sample is dissolved in 15% ethylenediamine–85% pyridine. The titrant is sodium dissolved in ethanolamine and diluted with pyridine. The indicator is thymol blue (*97*). Procaine and *p*-aminobenzoic acid are coupled after diazotization with 1-naphthylamine; after the dye formation is completed, the derivatives are separated on the basis of their solubility in acids (procaine) and bases (*p*-aminobenzoic acid) (*265*). The ultraviolet absorption maximum of procaine hydrochloride at 290 mμ has also been used to determine the amount of this compound in aqueous solution (*35*). A technique for the titration of reineckate salts in which reaction with the thiocyanate group is utilized offers a convenient method for the evaluation of certain organic bases (*161*).

Isotope dilution techniques have been applied to the assay of certain sulfonamide preparations utilizing sulfur-35–labeled sulfonamides. Ion exchange separations with selection of the peak fractions of the eluates for assay have provided suitable methods for determination of sulfanilamide and sulfadiazine in mixtures (*191*). Sulfonamides have been titrated in pyridine solution with sodium methoxide; tablet excipients interfere (*115*). Identification of sulfonamides has been accomplished by paper chromatography (*310, 394*) and by paper ionophoresis (*390*). Tetracaine is separated from its hydrolytic products by extraction above pH 8 with an organic solvent; its concentration may be measured by ultraviolet absorption techniques (*106*); the authors have described in the same paper a colorimetric assay for phenylephrine. A volumetric determination of thiouracil and certain of its alkyl homologs has been developed by Abbott (*1*); the titrant is mercuric acetate and the indicator is diphenylcarbazone. Interferences from tablet excipients are few and can be avoided. A specific color reaction for compounds of the thiouracil type has been described, and a method of determination of propylthiouracil is given (*234, 235*); the reaction involves condensation with 2,6-dichloroquinonechloroimide in a suitable buffered solution. A yellow pigment formed is extractable into chloroform. Systems for the paper chromatographic separation and identification of thiouracil and of methylthiouracil and propylthiouracil are butyl alcohol–water and amyl alcohol–water; zones are developed with iodine or by reaction with 2,6-dichloroquinonechlorimide (*225*).

GLYCOSIDES

Certain plant glycosides have been identified by means of paper chromatography. Location of the glycoside zones was achieved through enzymatic hydrolysis to produce a reducing sugar, which was then identified by spraying the strip with aniline hydrogen phthalate solution and heating. The glycosidases were applied to the chromatograms in a "strip transfer" technique. Such naturally occurring glycosides as amygdalin, arbutin, and salicin were identified (*40*).

Considerable information concerning the identification and determination of the digitalis glycosides has appeared in the recent literature. Several of the primary glycosides of digitalis have been separated and identified by paper chromatography in an ethyl acetate–ethanol-benzene-water system; fluorescent zones were formed by spraying the chromatograms with trichloroacetic acid and heating. The system is not satisfactory for the separation of desglucoglycosides such as gitoxin and digoxin (*338*). A colorimetric method for the estimation of digitoxin utilizes reaction of the glycoside with 3,5-dinitrobenzoic acid in the presence of benzyltrimethylammonium hydroxide to produce a measurable color. The absorption maximum of the chromophore is 550 mμ. The method has the advantage of a very low reagent blank, but the color produced is fleeting. Digoxin and gitoxigenin also react at a slightly different rate (*293*). An investigation of the alkaline picrate method for digitoxin determination has indicated an effect attributed to the presence of ethanol in the system. The same article describes a process for decolorizing digitalis tinctures prior to color development (*314*). Hassall and Lippman (*167*) have utilized a reaction of digitalis with *m*-dinitrobenzene at pH 11 to 13 to produce a red color. The colored product is said to be more stable than the transient blue colors obtained with stronger alkali. The procedure has a rather low sensitivity for digitoxin.

A fluorometric procedure for determination of the aglycone of digitoxin has been reported by Jensen (*197*). The sample, dissolved in 8.4*M* hydrochloric acid, is oxidized with a small amount of hydrogen peroxide; digitoxigenin forms a compound exhibiting blue-green fluorescence. Further reaction is prevented by the addition of ascorbic acid. Gitoxigenin, gitoxin, and purpurea glycoside B have only weak fluorescence; digitoxin in the range of 2 to 20 γ in 10 ml. may be determined with an accuracy of $\pm 5\%$. Chromatographic separation of digitoxin and digitoxigenin has been achieved on alumina columns. The aglycone is eluted with chloroform while 3 to 1 chloroform-ethanol elutes digitoxin (*201*). Paper chromatographic separation of digitalis glycosides has been investigated by several workers. The most satisfactory systems appear to be those utilizing formamide-impregnated paper with an organic mobile phase consisting of chloroform or benzene or mixtures of these (*198, 323*). Other systems described include butanol–amyl alcohol–water (*157*) and chloroform–ethyl acetate–benzene-water (*339*); the latter system is suitable only for very small quantities (1 to 5 γ). An interesting study of "new" and "aged" solutions of trichloroacetic acid in chloroform and their effect on the development of fluorescence with digitalis glycosides has been made (*339*); these workers observe that a freshly prepared reagent gives no fluorescence with digitoxin, gitoxin, and digoxin. The

Carr-Price reagent has been suggested also as a color reagent for identifying digitalis glycosides on paper chromatograms. The colors produced are reported to be permanent and distinctive (*223*). Digitoxin tablets have been analyzed by partition chromatography in which the stationary phase is Celite impregnated with formamide-water. The mobile phase is chloroform-benzene. Digitoxigenin is eluted first from the column, followed by digitoxin; gitoxin stays on the column (*17*). These authors point out that the U.S.P. XIV monograph for digitoxin permits acceptance of material which could contain as little as 70% of the glycoside. The presence of appreciable amounts of gitoxin in commercial samples of U.S.P. digitoxin was demonstrated.

The possible use of ion exchange resins to isolate glycosides as borate complexes was investigated by Chambers *et al.* (*76*). It was found that the glycosides were not retained on strong anion exchangers; however, it may be possible to remove contaminating sugars by this technique. Extensive studies on the chromatographic, ultraviolet absorption, and color-forming properties of digitoxin have resulted in the conclusion by DeMoen and Janssen (*93*) that the true physical and chemical properties of digitoxin are still largely unknown. Much of the discrepancy observed between the U.S.P. XIII pigeon assay and the U.S.P. XIV colorimetric assay for digitoxin has been attributed to the high gitoxin content of commercial digitoxin (*47*).

The U.S.P. XIV colorimetric method for determination of digoxin has been modified by Banes (*15*). The modification involves the use of weaker alkali (1% tetramethylammonium hydroxide). The result is slower development of color with absorption maximum at 615 mμ. Gitoxin develops a feeble red-violet color, which fades rapidly and does not interfere when the gitoxin content is less than 8% of the total glycoside present. Gitoxigenin has been determined fluorometrically by dissolving the sample in 1 to 1 concentrated hydrochloric acid–glycerol. After 20 minutes, the fluorescence developed may be used to evaluate the gitoxigenin content quantitatively. Digitoxin, digitoxigenin, and purpurea glycoside A do not form fluorescent compounds under these conditions. The procedure is sensitive to 1 to 10 γ in 10 ml. (*195*). A colorimetric estimation of gitoxigenin in the presence of digitoxigenin (*371*) involves reaction of the aglycone with ferric chloride in a sulfuric acid–phosphoric acid mixture. The paper chromatographic separation of gitoxin, gitoxigenin, and purpurea glycoside B has been observed in a chloroform-methanol-water system. The conditions of separation are very highly temperature-dependent (*196*).

A colorimetric determination of strophanthidin involves reaction of this glycoside with sulfuric acid. The minimal range of concentration is 0.5 to 1 γ per ml. (*147*). The aglycones of the strophanthus glycosides have been separated and identified by paper chromatography, using an ascending benzene–60% methanol system (*70*).

HORMONES, RELATED STEROIDS, AND STEROLS

One of the most complex and least well-defined classes of compounds of pharmaceutical interest are those involving the cyclopentanoperhydrophenanthrene nucleus. Many of these compounds such as the bile acids, cardiac glycosides, veratrum alkaloids, and certain of the sex hormones have been well known for some time. Considerable effort has been expended in the development of analytical methods for these materials, few of which are completely satisfactory; the situation is even less encouraging for the newer and more recently investigated steroids. Much of the difficulty associated with the determination of specific compounds may be attributed to the marked similarity of associated steroids so far as physical and chemical properties are concerned. Several techniques, however, appear promising for purposes of separation of closely related steroidal materials.

Partition chromatography has been used successfully in the separation of the active adrenocortical hormones. The stationary phase in the particular system described is water–silicic acid and the mobile phase is petroleum ether with increasing amounts of dichloromethane. Eluted fractions are identified by reaction with concentrated sulfuric acid, and quantitative assay of the fractions is accomplished by a measurement of ultraviolet absorption (*176*). A quantitative paper chromatographic method for the evaluation of adrenal extracts in which the reducing cortical steroids are identified on the paper chromatogram with arsenomolybdate reagent has been developed by Schwartz (*329*). The blue zones are eluted from the paper and absorption of the complex is measured photometrically. Steroids with a ketol side chain or an α,β-unsaturated 3-keto structure quantitatively reduce the reagent. Banes (*14*) has separated corticosteroids in adrenal extracts by a partition chromatographic method; in this case, the stationary phase is formamide in Celite, while the mobile phase is benzene. Infrared measurements are used to identify individual steroids in the eluate fractions. In the sulfuric acid–induced fluorescence reactions of corticosteroids, Sweat (*363*) has made a critical study of the assay conditions.

Certain Δ^4-unsaturated-3-ketosteroids including cortisone acetate, progesterone, and testosterone develop strong stable absorption maxima in the near ultraviolet range when heated with dilute organic or inorganic alkalies (*87*). A colorimetric method for determination of cortisone and related ketol steroids is based on the fact that C_{20} keto and C_{21} primary-OH have reducing properties similar to fructose. Alcoholic solutions of these steroids reduce tetrazolium salts in the presence of tetramethylammonium hydroxide to form colored solutions (*239*). Cortisone has been observed to react with the anthrone reagent to produce a color measurable at 480 mμ; the optimum concentration range is 0.5 to 2.0 mg. per ml. of cortisone or cortisone acetate (*189*). A colorimetric method for the determination of diethylstilbestrol and related estrogens is reported by Kelly and James (*205*). Other methods for determination of diethylstilbestrol include an ultraviolet absorption difference procedure in which the quantitative measurements are made on acid and alkaline solutions of this compound and the difference at specific wave lengths is associated with changes in the concentration (*149*). The same report includes a new color reaction of diethylstilbestrol, in which the compound in acetic acid solution develops an intense yellow color when exposed to strong ultraviolet irradiation; if conditions are carefully controlled, the reaction is reproducible and may be applied quantitatively to the determination of this drug.

A detailed investigation of the ultraviolet absorption characteristics of ergosterol and a method for the determination of this substance in yeast are reported by Shaw and Jefferies (*335*). A collaborative study of several extraction and color development procedures for conjugated estrogens has been reported by Gaunt (*137*). Antimony trichloride dissolved in nitrobenzene has been reported to give characteristic colors with a large number of steroids. Separation of estriol, estrone, and α- and β-estradiol in a toluene–propylene glycol system on paper chromatograms may be followed utilizing the reagent described to identify the zones (*313*). Some sources of error have been discovered in the colorimetric determination of estrogens by the Kober reaction. Very extensive studies have been carried out to show the inhibitory effect of certain solvent residues on the color development. Residues from ether evaporation were shown to inhibit color development by as much as 15 to 40%. A modified reaction which is unaffected by solvent residues has been developed (*21*). A chemical method for the estimation of estrogens is based on the fluorescence developed when these compounds are heated in sulfuric acid (*384*). A reaction of estrone, estradiol, and estriol noted by Nyc *et al.* (*262*) may be of some analytical significance. These workers report that the compounds mentioned are destroyed at a rapid rate when exposed to visible light in the presence of riboflavin or lumichrome. Destruction is inhibited by the addition of small amounts of manganous chloride; the photo-oxidation results in a large loss of biological activity. A simple partition chromatographic procedure for separating the estradiols and dihydroequilin from complex estrogenic mixtures has been described (*158*, *159*), while estrone, estradiol, and estriol may be separated by partition chromatography also (*37*). The estrogens have likewise received much attention in paper chromatographic procedures. Among the systems used for separation are propylene glycol–acetone (*46*), formamide, benzene, or chloroform or their mixtures, methylene chloride, or cyclohexane (*9*, *418*), and chloroform-benzene-ammonia (*178*).

A colorimetric assay for pregnandiol involves oxidation at 0° C. with chromate, extraction of the ketosteroid with chloroform, and color development utilizing the Zimmermann reaction (*92*). The separation and quantitative determination of progesterone and pregnenolone are accomplished by forming the dinitrophenylhydrazones, which are separated on alumina and estimated spectrophotometrically (*303*). Progesterone has also been separated paper chromatographically and estimated by elution from paper, ultraviolet absorption measurements being made on the resultant solution (*104*). Testosterone propionate in vegetable oil solution is separated by quantitatively precipitating the semicarbazone. The derivative may be weighed directly or dissolved and the concentration determined spectrophotometrically at 268 mμ. Low potency oil solutions require a preliminary extraction from petroleum ether solution with aqueous ethanol (*240*).

Tauber (*372*) has developed a new color reaction for steroids in which chloroform solutions of the compounds are heated with perchloric acid to develop characteristic colors. Most of these solutions show intense fluorescence in ultraviolet light. The fluorescent reactions of steroids on treatment with concentrated sulfuric acid have been studied; emission peaks and relative peak intensities for 29 steroids are reported (*148*). The reactions of certain unsaturated steroids with a reagent composed of ferric chloride in glacial acetic acid diluted with concentrated sulfuric acid have been investigated by Zak *et al.* (*417*). Solubility analysis has been successfully applied to the determination of the purity of steroids. The technique has been extended to the identification of unknown impurities by a procedure somewhat analogous to mixed melting point processes and to the preparation of small quantities of pure steroids for use as reference standards (*370*). Bromoform and methylene bromide have been found to be good solvents for certain steroids which are difficultly soluble in carbon disulfide. Infrared absorption spectra of the compounds in these solvents are superior to those obtained in chloroform or carbon tetrachloride (*369*). Alumina-impregnated paper has been found suitable for adsorption chromatographic separation of steroids using an ascending organic solvent, while a partition system consisting of petroleum ether–aqueous methanol has been applied likewise in separating steroids on paper (*69*).

A rapid method for total cholesterol estimation in serum permits the direct addition of reagents to a small serum sample followed by absorptiometry of the color developed (*275*). The modification of the Liebermann-Burchard reagent by the addition of 1,4-dioxane reduces the rate of color development so that the reaction can be run at room temperature (*233*). A slightly different color reagent for cholesterol is 10 to 1 acetyl chloride–concentrated sulfuric acid (*377*). A paper chromatographic separation of certain sterols together with a description of indicator spray solutions or dipping solutions is reported by Neher and Wettstein (*259*). It has been observed that sapogenins dissolved in 94% sulfuric acid give characteristic spectra in the 220 to 400-mμ range. A quantitative scheme based on chromatography, spectra, and melting point for 13 common sapogenins is described (*392*). Wall *et al.* (*393*) have developed a procedure for detection and estimation of steroidal sapogenins in plant tissues. A positive hemolytic test on alcoholic plant extracts indicates the presence of saponins; samples showing a positive test are hydrolyzed and acetylated and a carbon disulfide or chloroform solution of the extracted residue is subjected to infrared analysis to determine the type and amount of saponin present. A paper chromatographic method for the separation of steroidal sapogenins and their acetates has been reported (*175*). The separated sapogenins are identified by spraying with trichloroacetic acid or blood.

METALLIC IONS AND SIMPLE ANIONS

Analytical methods for the determination of simple cations and anions such as metallic substances, halides, and related simple ionic species are as much a part of the pharmaceutical analyst's field of interest as any group of organic substances. The increasing emphasis on the physiological effects of trace metals and ions is extending the control effort as well as the investigative effort of the analyst to the microgram range. Consideration must be made also of the many opportunities for simplification and improvement in specificity and economy which the more recent techniques offer.

A particularly useful application to the analysis of insoluble salts of pharmaceutical interest has been described by Brochmann-Hanssen (*52*). The batch treatment of such substances as calcium mandelate, some of the calcium phosphates, calcium citrate, lead chloride, barium sulfate, bismuth subnitrate, and bismuth subsalicylate, with a cation exchanger in the hydrogen cycle results in the exchange of hydrogen ion for the cation of the insoluble salt. The soluble acid thus produced may be separated from the exchanger and titrated. Qualitative information on the infrared spectra of 159 inorganic compounds has been obtained (*254*). Curves of the compounds—principally salts of polyatomic ions—were obtained from mulled samples in Nujol or Fluorolube or directly from powder. In some solvent extractions an error is introduced by reason of the solubility of certain inorganic compounds in the organic solvents used for extraction; quantitative data on the solubility of several commonly occurring inorganic salts in such solvents as methanol, ethanol, acetone, ethyl acetate, and alcohol are reported (*328*).

An interesting concept of the interaction of Lewis acids and bases may be of potential pharmaceutical interest. Certain aprotic acids such as stannic chloride, aluminum chloride, and aluminum bromide were successfully titrated in a thionyl chloride medium with pyridine as a titrant (*136*). Indicators used were crystal violet and malachite green; stannic chloride was titrated conductometrically with benzophenone. A new separation step in the determination of such substances as phosphorus, vanadium, silicon, and germanium involves extraction of the heteropoly complexes of these elements into organic solvents (*389*); the ultraviolet absorption curves of the complexes thus isolated from the excess molybdate in the solution may then be utilized for quantitative evaluation.

The development of adequate qualitative and semiquantitative tests for heavy metals in pharmaceuticals continues to receive well-deserved attention. Stewart and Strode (*357*) have devised a scheme involving carefully standardized conditions, for comparing sample colors in the heavy metals test through photoelectric measurements. Another approach to the elimination of the visual differentiation of nonstandardized colors utilizes amperometric titrations for the determination of microgram quantities of heavy metals. An accuracy of 0.5 p.p.m. in the range 1.5 to 10 p.p.m. in organic drugs is claimed for such metallic ions as copper, silver, lead, mercury, and cadmium (*86*).

A very sensitive procedure for the colorimetric determination of free ammonia is described by Kruse and Mellon (*218*). The method involves measurement of the purple color formed when the sample is treated first with dilute chloramine-T, followed by a pyridine solution of phenylmethylpyrazolone. The color formed may be extracted into carbon tetrachloride and the absorbancy measured at 450 mμ. As little as 0.025 p.p.m. of ammonia may be detected. The method has been extended to the determination of cyanate ion by hydrolysis to ammonia with subsequent color development (*217*). An improved method for the volumetric determination of arsenic involves the reduction in solution of arsenic by hypophosphorous acid (*166*); the resulting elemental arsenic is titrated iodometrically. The method has been applied to the determination of arsenic in arsenite solutions and is applicable in the presence of antimony. Another colorimetric method for arsenic determination involves reaction of arsenic with a pyridine solution of $AgSCSNEt_2$ to produce a red color. Sensitivity is reported to be 0.5 γ of arsenic. Stibine develops a different shade of color, while phosphine does not interfere (*383*). Bricker and Sweetser (*51*) have utilized ultraviolet absorption changes in the solution for a photometric determination of the end point in titrating micro quantities of arsenic. Amperometric titration of bismuth compounds with 8-quinolinol has been applied to several official bismuth compounds satisfactorily (*39*). A method for determining cadmium is based on the nephelometric measurement of a precipitate of the iodocadmiate of brucine at pH 5 (*116*).

With the increased use of highly sensitive photomultiplier tubes as detecting elements for radiation, the direct determination of the alkali metals by flame photometry has increased in practical application. The advantages of the narrow slit widths which are possible with such detectors in determining calcium have been described (*207*). Some additional applications of the disodium dihydrogen ethylenediamine tetraacetate titration for the determination of calcium in several organic medicaments without the necessity of preliminary separation have been reported (*227*). A scheme for the successful separation and absorptiometric determination of copper, manganese, and iron may be applied to 10 to 20 γ quantities of each substance (*414*). A critical study of equilibria in the dithizone-water system for the copper complex of dithizone is described in terms of the analytical significance of the results (*139*). A new specific reaction of cuprous ion with 2,9-dimethyl-1,10-phenanthroline (neo-cuproine) has been observed by Smith and McCurdy (*347*). The complex, soluble in higher alcohols, exhibits an absorption maximum at 454 mμ. Ferrous ion does not interfere.

A potentially useful separation procedure for the removal of iron in complex mixtures employs an extraction of the thiocyanate complex into an organic solvent. At pH 2.1, 95% of the iron in a 0.01M aqueous solution containing excess thiocyanate ion can be removed in a single extraction with 1 to 1 tributyl phosphate–carbon tetrachloride (*249*). The extracting solvent has the advantage over butyl phosphate alone of being heavier than water. A procedure for the simultaneous determination of lead, bismuth, and thallium as the halide complexes has been devised for concentrations of the order of 10 p.p.m.; ultraviolet absorption curves of the complexes are used for quantitative measurement (*251*). It has been found possible to titrate lead solutions directly in the presence of added tartrate to prevent hydroxide precipitation; after adjustment to pH 8 to 10 with aqueous ammonia, disodium ethylenediamine tetraacetate is added as the titrant. Zinc, copper, and nickel interferences are eliminated by addition of potassium cyanide; calcium and magnesium interfere (*119*). The slightly dissociated red complex of magnesium with Eriochrome Black T is the basis for a colorimetric determination of magnesium. Interfering calcium ion is removed by precipitation as calcium sulfate from 90% methanol solution (*165*). Mercury has been titrated also with disodium ethylenediamine tetraacetate using Eriochrome black T as indicator (*295*). A critical evaluation of the thiocyanate titration of mercury for several pharmaceutical products has been made by Cumming and Spice (*88*).

A quantitative paper chromatographic method has been applied in the direct determination of nickel in microgram quantities; after development in an acetone–hydrochloric acid system, the chromatogram is sprayed with rubeanic acid and dried and the total density of the nickel spot read by reflectance measurements in a spectrophotometer. The precision is reported to be equal to that of the best colorimetric methods (*380*). The alkali metal sulfates and citrates have likewise been separated on paper chromatograms; sodium, lithium, and potassium sulfates are detected with a spray of barium chloride in 70% ethanol followed by sodium rhodenionate solution. Methanol, ethanol, and acetone mixtures were used as solvents (*68*). Pifer *et al.* have demonstrated a significant difference in basicity between potassium and sodium ions in acetic acid–chloroform solution. By potentiometric nonaqueous titration, it is possible to differentiate these ions quantitatively in the presence of each other (*284*). A rather specific separation technique for the isolation of silver ion involves precipitation as the 1,2,3-benzotriazole complex from ammoniacal Versene solution. The precipitate dissolves in the presence of excess cyanide or thiosulfate (*80*).

A micromethod for the determination of halogens in organic compounds is reported which involves fusion with sodium, ethanol washing, and finally a potentiometric titration (*257*). Organically bound iodine may be determined in a mixture after digestion with potassium chloride and perchloric acid; the iodine present in the resulting solution as iodate may be estimated volumetrically, spectrophotometrically, or polarographically. The method has been used for the determination of iodine in thyroid extract (*416*). In an analytical method for iodine and iodide ion in tincture of iodine, the free iodine is adsorbed on charcoal and removed by filtration. Iodide in the filtrate is then titrated with silver nitrate, while the free iodine may be titrated with sodium thiosulfate solution (*355*). A titrimetric microdetermination of alkali halides involves illumination of a neutral solution of the halide with a narrow beam of light while it is being titrated with silver nitrate solution; at the equivalence point, the dimensions of the colloidal particles of silver halide suddenly increase; and the color of the transmitted beam changes sharply from colorless or yellow to red (*168*). By utilizing the reaction of chloride ion with silver iodate to release an amount of iodate ion equivalent to the amount of chloride ion present, Belcher and Goulden (*23*) have increased the apparent titer for chloride ion sixfold; the released iodate ion will require six equivalents of sulfite ion as titrant for each chloride ion present.

Elemental sulfur may be determined by dissolving the sample in ethanol and measuring the ultraviolet absorption of the resulting solution. Up to 40% water in ethanol has no effect on the absorption. Other sulfur compounds and proteins interfere and must be removed (*174*). A method for the determination of cyanates involves colorimetric measurement of the dicyanatopyridine copper(II) complex in chloroform solution. The method is applicable to 0.5- to 5-mg. quantities (*246*). A procedure for the quantitative determination of nitrates utilizes the principle of enzymatic reduction of nitrate to nitrite followed by a colorimetric determination of the nitrite ion. The method is applicable for the determination of extremely small quantities of nitrate in body fluids (*360*). A method of separating nitrite from nitrate ion involves reaction to produce very volatile methyl nitrite. The product can be absorbed by a cold aqueous saturated solution of ferrous sulfate in sulfuric acid with formation of a characteristic brown coloration. Nitrate ion will remain behind (*127*). Microgram and smaller quantities of phosphate have been determined spectrophotometrically in capillary cells. The absorbing complex is phosphomolybdic acid, which is extractable from aqueous solution with octyl alcohol (*322*).

A relatively simple gravimetric determination of sulfate without the use of agents for increasing crystal size and without other additives eliminates interference from foreign ions by means of ion exchange resins; smaller amounts of sulfate may be determined by instrumental methods (*129*). Nydahl and Gustafsson (*263*) have found that alkali-washed alumina retains certain anions such as sulfate, bisulfate, and acid phosphate; these may be eluted with sodium hydroxide solution. A colorimetric method for determination of thiocyanate and cyanide ion involves absorption measurements of a stable complex of the respective ions with copper and pyridine in chloroform (*216*).

PROTEINS, AMINO ACIDS, AND RELATED COMPOUNDS

The application of the newer separation and measurement techniques to the identification and determination of proteins has received much emphasis in recent years. Ultraviolet absorption measurements of the albumin and globulin fractions of serum proteins have indicated reproducible extinction values in the 270- to 300-mμ range (*252*). Comparative results between ultraviolet absorption measurements at 275 mμ and micro-Kjeldahl analysis show satisfactory agreement in evaluating the salting-out curves of proteins. The spectrophotometric method is especially convenient for work with ammonium sulfate (*94*). A micromethod for evaluation of fibrinogen involves precipitation with sodium chloride; the turbidity is measured nephelometrically (*140*). A gravimetric estimation of proteins precipitated by trichloroacetic acid is reported by Hoch and Vallee (*184*). Good agreement among weight of crystalline protein, absorption values at 280 mμ, and weight of a properly dried residue from trichloroacetic acid precipitation was obtained. Among the samples tested were pure human and bovine serum albumin, pure bovine insulin, and crude bovine pituitary extract. Molecular weight does not appear to be a determining factor in precipitation by trichloroacetic acid of proteins of weight greater than 12,000.

Paper chromatographic techniques again appear to be potentially useful in separation procedures for proteins. The behavior of proteins and peptides in partition chromatography with phenol-containing solvents has been investigated (*153*). In many instances, phenol had an excellent effect on separation or mobility of different proteins. Franklin and Quastel have compared paper chromatograms of normal and pathological plasmas (*126*). The mode of transport of protein mixtures by ascending techniques has been studied (*160*). Paper electrophoretic separations of proteins continue to receive attention. A study of variables including the dye-binding capacity of different protein fractions has been made by Hardewicke (*162*). A quantitative accuracy within ±6% of the total protein present is claimed. A source of error in the quantitative determination of albumins by filter paper electrophoresis has been demonstrated to be an adsorption phenomenon which increases with the thickness of the paper employed (*250*). Variation in degree of adsorption among different types of paper was noted.

The separation of enzymes and their identification by paper chromatography have been reported (*144*). Amylases, phosphorylase, and phosphatases have been separated on Whatman No. 1 filter paper with solvents such as aqueous acetone and dilute sodium chloride; the positions of zones were noted by plating on agar substrates and incubating. Activity was located by changes in indicators included in the substrate. The paper chromatographic separation of amylase in commercial trypsin powder has been achieved in aqueous acetone or sodium chloride systems (*143*). A turbidimetric method for estimating esterase activity employs blood serum containing acetylcholine and 0.1% casein at pH 8 for a substrate. The opalescence developed in the solution, as measured at 650 mμ, is shown to be a function of the amount of acid released by hydrolysis of the ester (*290*).

Porter (*291*) has investigated the purity of insulin prepared from crude or crystalline material by partition chromatographic methods. Three systems containing water, ethyl and butyl Cellosolves, and sodium or potassium phosphate were utilized. Recovery from the columns appeared to be nearly quantitative. Insulin has been separated from protamine by paper chromatography in a butanol–acetic acid–water system. A dye such as bromocresol green is used for locating the zones. The insulin-dye complex can be eluted from the paper and compared to standards (*309*).

The separation and evaluation procedures for peptides, nucleic acids, amino acids, and related substances have proceeded along more or less conventional lines. Paper ionophoresis has been employed in the separation and determination of ribonucleotides. Identification and measurement of compounds were accomplished by a manual scanning procedure using a Beckman spectrophotometer equipped with a high intensity light source (252 mμ) (*401*). The total α-amino nitrogen in a sample containing amino acids can be accurately determined by measuring the carbon dioxide released by ninhydrin. Recoveries of 97 to 102% were obtained with amounts corresponding to about 150 γ of α-amino nitrogen (*343*). A new color method for the detection of amino acids, peptides, and proteins on paper has been developed (*305*); treatment of the paper with chlorine results in formation of chloramine derivatives which in turn react with benzidine to produce stable, intensely blue spots.

Of special interest is the proposed optical resolution of certain aromatic amino acids on paper chromatograms. For stereochemical specificity to be realized, specific structural requirements are stipulated. Included in these is a "three-point" attachment to the cellulose surface (*90*). Various quantitative paper chromatographic methods for the separation and determination of amino acids in mixtures are reported in the recent literature (*61, 141, 183, 274, 301, 312, 317*). Displacement chromatography on ion exchangers has been used also in the separation and determination of amino acids (*273*). Circular paper chromatography of amino acids has been applied by Saifer and Orespes (*316*). These authors have applied isatin as a color reagent; 12 of 21 amino acids tested gave distinct color reactions. In applying fluorodinitrobenzene to the quantitative analysis of proteins, Mills (*255*) has employed columns of kieselguhr developed with mixtures of methyl ethyl ketone and chloroform saturated with water to distinguish the dinitrophenyl derivatives of the various amino acid constituents.

Several methods for the determination of thyroxine have been investigated with special reference to tablet mixtures. Of several known procedures, a colorimetric method involving treatment with nitrous acid and ammonia appeared to be the best (*3*). An interesting observation on the resolution of *dl*-tryptophan by paper chromatography has been made (*82*); the racemic mixture showed two spots on unbuffered chromatographic paper. These were identified as D- and L-tryptophan, respectively. Results seem to indicate that resolution on filter paper is accomplished only with amino acids bearing two or more polar groups. The separation of glycine from gelatin has been accomplished by passing the sample solution over a mixed-bed resin. A formol titration performed before and after the ion exchange step permits determination of the free amino acid by difference. The procedure could be applied in following the hydrolysis of gelatin (*321*).

SUGARS AND CARBOHYDRATES

The determination of small quantities (17 to 65 mg.) of glycerol has been made possible by modification of the sodium cupri-glycerol complex method of colorimetric analysis (*351*). Extending a method previously used for ethanol determination, Reese and Williams (*302*) have determined glycerol by oxidation with dichromate solution. The reduced chromium(III) ion thus produced is complexed with *s*-diphenylcarbazide to form an intense violet color. The very high dilution afforded by the estimation of the chromium(III) eliminates interferences from dichromate ion. Simple, rapid methods for glycerol determination, reported by Erskine *et al.* (*110*) and Hartman (*164*), involve periodate oxidation of the compound. A chromatographic separation for the removal of interferences in determining glycerol in fermentation solutions has been described (*352*). In this case an alumina adsorbent is used to hold up interfering sugars while glycerol is eluted and subsequently reacted with sodium metaperiodate. The developing solvent is acetone containing 5% water and 0.05% acetic acid. Sodium sulfite and sodium acetate are added to the sample solution.

A specific color reaction for sugars has been recently proposed by Tauber (*373*). The test involves reaction of the sugar solution with a solution of aminoguanidine sulfate, followed by oxidation with potassium dichromate in concentrated sulfuric acid. Ketohexoses give an immediate wine-red color, aldohexoses give a deep blue color in 1 minute, and pentoses give a yellow color. A uniquely useful process for the separation of various sugars has been developed by Khym and Zill (*206*), who have produced negatively charged sugar-borate complexes in solution. The anions may then be exchanged on strong base anion exchangers. Serial elution of the complexes from the resin columns is accomplished with dilute sodium borate solutions. The recovery of individual sugars was essentially quantitative. Improved methods for the detection of sugars on paper chromatograms include a sodium borate–phenol red reagent for direct spraying of sugars on paper (*185*). It is also possible to use unneutralized sugar hydrolyzates for paper chromatography, if a spray solution of aniline phosphate or phthalate is used (*134*). Circular paper chromatography has been employed in the separation and identification of a large number of sugars in a mixture (*142*). Fowler has developed a quantitative paper chromatographic technique for the estimation of sugars. A quantitative relationship between zone length and zone content has been shown to exist in the range 4 to 450 γ and a study of variables is reported (*123*).

The reducing power of fructose and sorbose has been utilized in a reaction with the Folin-Denis reagent and trisodium phosphate. The color can be used in determining the amount of sugar in the solution with an accuracy of about 2% (*163*). A very specific method for the determination of glucose involves oxidation of this compound to *d*-gluconolactone by glucose dehydrogenase; the lactone may be titrated with alkali at elevated temperature (*403*). A rapid procedure, reported to require only 5 to 10 minutes, for the determination of glucose or other hexoses in biological fluids involves reaction with *o*-dinitrobenzene in alkaline alcoholic solution (*280*). Frampton *et al.* (*124*) have adapted a cyanohydrin formation reaction of aldoses with hydrogen cyanide to the quantitative determination of these sugars. The nitrile, formed at pH 8.5, is hydrolyzed to release ammonia, which is trapped and determined titrimetrically. The quantitative oxidation of aldoses to aldonic acids by hypoiodous acid is the basis for an analytical procedure for the determination of mannose and other aldoses (*83*). Dische (*96*) has reported qualitative and quantitative techniques for the identification and determination of heptoses.

Utilizing differential solubilities, methoxyl determinations, and the colorimetric anthrone method, fairly accurate estimations of the methylcellulose content of mixtures can be achieved (*318*). Similarly, Black (*38*) has employed the anthrone reagent in the determination of sodium carboxymethylcellulose. Other carbohydrates and certain polyoxyethylene derivatives of fatty acids and phenols interfere. The intensity of color produced with carboxymethylcellulose varies inversely with the degree of substitution of the material. A method of colloid titration applied to the determination of sodium carboxymethylcellulose is described by Terayama (*375*). Titrations are based on stoichiometric combinations of positive and negative colloid ions. The end point is shown by indicators such as toluidine blue, giving metachromic colors. A rapid (2 to 4 hours) method for the determination of sodium carboxymethylcellulose involves the formation of an insoluble salt with uranium ion. Minimum recovery of 98% has been demonstrated on a variety of samples (*125*).

Gluconic acid, a product of the oxidation of glucose with glucose oxidase and catalase in the presence of hydrogen peroxide, can be chromatographed on paper using an aqueous methanol system. Identification is made by spraying the paper with ferric chloride. The acid shows up as a light yellow zone on a mauve background (*331*). Formaldehyde produced by periodate oxidation of carbohydrates may be colorimetrically determined with chromotropic acid (*264*). The method has been applied to monosaccharides, polyhydric alcohols, serine, and *N*-acetylglucosamine.

An excellent comprehensive report of the physical and chemical properties of clinical dextran has been published by Riddick *et al.* (*308*). Analytical methods for the various physical and chemical specifications are described. Satisfactory average molecular weights are determined by light scattering photometry and viscometry. Fractional precipitation with methanol permits the evaluation of the molecular weight distribution. Included is a method for the determination of methanol in dextran.

VITAMINS

Water-Soluble. Combinations of certain vitamins of the B complex have been separated by paper chromatography; thiamine, riboflavin, and pyridoxine after having been chromatographed in the system 1-butanol saturated with water, are eluted from the paper and the solutions evaluated spectrophotometrically. An accuracy within ±5% is claimed (*299*). Using the system 1-butanol–acetic acid–water, Brown and Marsh (*60*) have separated vitamins B_1, B_2, and B_6, and nicotinamide. Quantitative analysis of these mixtures was accomplished by a direct scanning technique applied to the dried chromatograms. Paper chromatographic separations of B_1, B_2, niacin, nicotinamide, and choline have also been reported (*179*).

The reaction of thiamine with ninhydrin is reported to yield a stable yellow color. The pigment is extractable from saturated sodium chloride solution with higher alcohols (*356*). The nonaqueous titration of thiamine salts with perchloric acid is based on the relative basicity of the vitamin in glacial acetic acid. Chloride salts require preliminary treatment with mercuric acetate (*283*). An interesting new reaction of thiamine has been reported by Teeri (*374*), who has utilized the reaction of B_1 in pH 6.6 phosphate buffer with cyanogen bromide to produce a fluorescent compound which can be measured quantitatively. Thiamine has been separated from interfering fluorescent substances by paper ionophoresis (*130*). A paper chromatographic identification of vitamin B_1 is possible on the basis of R_f values in the system acetic acid–pyridine-water. The thiamine is visualized by reaction with dipicrylamine (*241*). The quantitative separation and determination of thiamine and its phosphate esters have likewise been accomplished by paper chromatography (*340*). Ultraviolet absorption of solutions from extracted segments of the paper strips provides the necessary quantitative comparisons.

It would appear that the present, highly specific fluorescent methods of determination of riboflavin are adequate. Relatively few reports have appeared in the recent literature concerning new or improved methods for the determination of this vitamin. Seagers (*332*) has reported a simple polarographic procedure which requires little pretreatment of the sample; however, the sensitivity does not compare with that of the fluorometric technique. Periodic acid oxidation of riboflavin has been made the basis for a chemical method of determination. The reaction requires 3 moles of acid per mole of B_2 (*405*).

The oxidation of pyridoxine and pyridoxal with selenium produces a compound which will undergo the König reaction with cyanogen bromide and sulfuric acid. Pyridoxamine will undergo this reaction only after treatment with sodium nitrite and hydrochloric acid followed by oxidation with selenium. Quantitative evaluation of mixtures requires initial removal of the interferences by adsorption on Decalso (*364*). Separation procedures for the active forms of vitamin B_6 have also been developed using paper chromatographic techniques (*348*) and paper electrophoresis (*341*).

The continued high interest in vitamin B_{12} is reflected in the very large number of contributions which have appeared in the analytical literature during the past 3 years. Some of the apparent confusion which exists is undoubtedly due to the existence of several active forms of the vitamin. Problems associated with the isolation of the minute therapeutically active amounts of the vitamin present in extremely complex mixtures has required extensive developmental work for almost every preparation encountered. The possibility that several inactive pigments can and usually do accompany vitamin B_{12} in natural mixtures further complicates the isolation and determination picture. Recent analytical purification schemes for liver extracts have included paper electrophoresis (*330*) in which B_{12} remains as a stationary zone at the point of application while other active compounds migrate toward the electrodes, and chromatography on alumina after the addition of potassium cyanide to the sample (*226*). The ability of vitamin B_{12} (cyanocobalamin) to react with a second cyanide ion to produce a purple dicyano complex (*410*) has been made the basis for a new specific assay for vitamin B_{12}; thus, Rudkin and Taylor (*315*) have utilized the absorbancy difference between the visible spectra of cyanocobalamin and the dicyano complex formed from the cyanocobalamin in a sample as a quantitative measure of the vitamin. Also incorporated in the procedure is a nearly quantitative extraction of the dicyano form into benzyl alcohol. This preliminary extraction permits the purification of fermentation broths to a point where spectrophotometric measurements can be made. Obviously, analogs of cyanocobalamin which may be present in a sample will be first converted to the cyano form, so that the method measures total cobalamins. The dicyano complex has been satisfactorily used for the measurement of vitamin B_{12} extracted from liver (*382*). A titrimetric procedure involving the reduction of cyanocobalamin with chromium(II) ethylenediamine tetraacetate complex has been reported by Boos *et al.* (*45*).

The incorporation into vitamin B_{12} of cobalt-60 by fermentation with *Streptomyces griseus* with a specific activity of 120 μc. per mg. has been reported (*346*). Using cobalt-60–labeled cyanocobalamin, Bacher *et al.* (*10*) have developed radioactive tracer assays for this and other cobalamins in mixtures. Their paper describes various purification steps, combinations of which may be used in isolating pure material from such preparations as fermentation broths and concentrates. Differential determination of cyano and total cobalamins is possible with this technique; the method can be made highly specific. Some precise studies of the purity of vitamin B_{12} have been made by Heathcote (*172*). Partition coefficients for the systems benzyl alcohol–water and butanol–water have been determined under carefully controlled conditions of temperature and pH. These values are helpful in determining the absence of analogs or associated inactive pigments.

Of some analytical import are the recommendations of Smith (*344*) and Smith *et al.* (*345*) concerning the classification and stability of the various analogs of vitamin B_{12}. Included in these studies is a refutation of the existence of "vitamin B_{12d}." In general, when negative ions with strong coordinating affinities are combined with cobalamin, neutral compounds are produced (cyanocobalamin and nitritocobalamin). Of considerable pharmaceutical interest is the problem of stability or lack of stability of cyanocobalamin and its analogs in the presence of ascorbic acid. Relative stability is a function of the coordination of anions with the cobalt present in cobalamin—e.g., sulfite, nitrite, and cyanide protect the vitamin from destruction (*132*). Some very interesting studies concerning the incorporation of small amounts of metallic ions with cyanocobalamin and ascorbic acid have been made by Stapert *et al.* (*354*). These workers have shown that the inclusion of small amounts of copper ion results in the rapid destruction of cobalamins—even of cyanocobalamin. The addition of cyanide ion or of sodium diethyldithiocarbamate reduced the decomposition of vitamin B_{12}. The data indicate that vitamin C alone does not readily affect either cyanocobalamin or its analogs, in contrast to earlier observations of the destructive action of ascorbic acid. It has been shown also that Versene has a stabilizing effect on vitamin B_{12} in the presence of ascorbic acid in the liquid vehicles (*20*).

The fact that hydroxocobalamin is readily destroyed in solutions containing ascorbic acid, while cyanocobalamin is relatively stable in such solutions, has been used as the basis of a procedure to determine hydroxocobalamin in the presence of cyanocobalamin. In the event that analogs other than hydroxocobalamin were present in the mixture, their relative rates of decomposition would contribute to the apparent hydroxocobalamin content of the sample (*73*). Another analytical technique for the determination of analogs in the presence of cyanocobalamin utilizes the differences in partition coefficient in the system benzyl alcohol–water. Concentration measurements for the vitamin in each phase are made at 356 mμ (isoabsorptive point) (*171*).

Of particular interest in the analysis of complex natural mixtures for vitamin B_{12} is the discovery of factors closely related to the vitamin and microbiologically active (*L. leichmannii*) but completely inactive in promoting growth in animals (*95*, *231*). At least two pseudovitamins B_{12} have been characterized. These appear to be similar to cyanocobalamin, differing only in the replacement of the 5,6-dimethylbenzimidazole moiety with adenine (*282*) and 2-methyladenine, respectively (*58*). That the remainder of the molecule is identical with cobalamin has been demonstrated by hydrolysis to yield denucleotided cobalamin from cyanocobalamin and from the two pseudovitamins as well (*135*). Similarly, Ford and Holdsworth (*121*) utilized adenine and 2-methyladenine as precursors in the production of the two pseudovitamins, while other precursors such as riboflavin, 4, 5-dimethyl-*o*-phenylenediamine, or 5,6-dimethylbenzimidazole produce vitamin B_{12}. The work of Ellis *et al.* (*107*) offers some analytical possibilities, particularly in connection with the formation of certain chlorinated products of vitamin B_{12}. One of these derivatives is reported to have an absorption maximum near 615 mμ, which would be outside the normal range of interferences encountered in spectrophotometric examinations of complex mixtures.

The color developed by the reaction of ascorbic acid with a concentrated uranium nitrate solution has been made the basis of a colorimetric method for the determination of this vitamin (*18*); the sensitivity of this procedure is relative low. Schmall, Pifer, and Wollish have described a new, specific reaction of ascorbic acid with diazotized 4-methoxy-2-nitroaniline in acid solution followed by alkalinization. The pigment produced has an absorption maximum at 570 mμ with $A^{1\%}_{1\text{ cm.}} = 430$ (*325*). Subsequent investigation (*326*) has resulted in the isolation and identification of the pigment. The elucidation of this complex reaction represents a considerable contribution to the explanation for the remarkable specificity of the procedure. Dehydroascorbic acid and diketogulonic acid oxidation products of vitamin C do not interfere nor do other vitamins when present in reasonable quantities. Dehydroascorbic acid has been converted to vitamin C, usually by reduction with hydrogen sulfide; a method for determining dehydroascorbic acid in which thioacetamide is employed in lieu of hydrogen sulfide has been described (*400*). The separation and estimation of ascorbic acid in complex systems have been accomplished by paper chromatography. Butanol-acid-water systems have been employed, the ascorbic acid zone being identified by starch-iodide reagent (*297*) or by ferric ammonium sulfate followed by *o*-phenanthroline (*41*). A quantitative paper chromatographic method involves elution from cutout segments of paper strips followed by color development in the extracts (*79*). Production of a fluorescent product by reaction of dehydroascorbic acid with *o*-phenylenediamine has been reported (*266*).

Chemical analytical methods for the determination of pantothenic acid and its salts have left much to be desired in terms of specificity. Among these relatively nonspecific procedures may be included a colorimetric assay based on the conversion of amides to hydroxamic acids by reaction with hydroxylamine (*29*). A considerable improvement in specificity appears to be possible with the recently developed procedure of Szalkowski and Davidson (*366*). In this method, hydrolyzed calcium pantothenate reacts with 2,7-naphthalenediol in the presence of sulfuric acid to produce a color complex with maximum absorption at 465 mμ. Interfering vitamins are removed by adsorption on Florisil and/or by reaction with calcium hydroxide and copper sulfate. Good agreement with microbiological assays is claimed.

A sensitive chemical test for choline involves the formation of an insoluble complex salt with sodium tetraphenylboron. Concentrations as low as 1 in 1 × 10^5 are detectable (*244*). A good possibility for an improved method for the determination of choline would appear to be the procedure developed by Appleton *et al.* (*8*) for plasma. In this technique, use is made of the extractability of choline periodide into ethylene dichloride. The compound has a distinctive ultraviolet spectrum which is different from that of iodine. Absorption maxima appear at 365 and 295 mμ. Low limit of determination is 5 γ of the base. Paper chromatographic separation of choline esters has been reported (*404*), while the separation of choline from betaine, methionine, and inositol has been shown to be possible through reineckate precipitation in alkaline solution (*13*).

Pharmaceutical analysis continues to suffer from the lack of a satisfactory method for the determination of small amounts of inositol in complex mixtures. For separation and determination of macro quantities of this material, an acetylation, solvent extraction by ethylene dichloride, and gravimetric determination have been employed. Sugars interfere and must be fermented. Polar materials are removed by ion exchange; choline, betaine, and methionine do not interfere (*12*).

In the reaction of nicotinic acid and nicotinamide with cyanogen bromide, it has been found possible to differentiate between these two substances in alkaline solution. Under alkaline conditions, the amide reaction product is fluorescent (*224*). A selective adsorbent for rutin and quercetin has been described recently. The material, which is bismuth hydroxide, adsorbs these substances from very dilute solutions; after conversion of the adsorbent to bismuth sulfide, rutin can be extracted from the precipitate with alcohol acidified with acetic acid (*415*).

Oil-Soluble. Specific solvent effects on the absorption spectrum of vitamin A and on the spectrum of all-trans vitamin A acetate have been reported (*44*). Precise spectrophotometric data have been obtained also for chromatographically purified vitamin A_2 in several solvents. The determination of this material in the presence of vitamin A is discussed (*72*). Prokhovnik has devised a simple "difference" formula for the Morton-Stubbs correction as used in the spectrophotometric determination of vitamin A (*298*). A very comprehensive comparison of physical and chemical methods with the biological assay of vitamin A has been made (*258*). The closest correlation with biological values was obtained using the ultraviolet absorption method with the Morton-Stubbs correction.

Recent investigations concerning the reaction of vitamin A with certain metal halides has led to the view that the actual color-producing reaction of vitamin A with antimony trichloride is due to antimony pentachloride, which is present as an impurity in the salt (*62, 64, 65*). It has been shown in these studies that the characteristic halochrome salt may be readily formed with vitamin A and very small quantities of the pure pentachloride. The formation of similar complexes with ferric chloride, aluminum chloride, arsenic trichloride, and arsenic pentachloride is discussed. The reactions of vitamin D and carotene with these same salts are also discussed.

Separation procedures for vitamins A, D, and E have received considerable attention in recent years. Most of these techniques involve either adsorption or partition methods. A convenient standardization of adsorbent mixtures for use in vitamin A separations is discussed by Wilkie and Jones (*408*). These authors treat in batch fashion a quantity of the adsorbent with a petroleum ether solution of F and D Butter Yellow No. 4 dye. Spectrophotometric measurement of the dye remaining in the solvent after contact with the adsorbent is used as a relative indication of the adsorptive power of magnesium oxide–Celite mixtures. The "adsorption index" so obtained is correlated with the chromatographic performance of the adsorbent for vitamin A. An adsorption technique has also been used for the separation of vitamin A alcohol from α-tocopherol. For this purpose, partially deactivated alumina columns have been used. The procedure is not satisfactory for the separation of vitamin A from mixed natural tocopherols nor is information concerning the performance of esters of vitamin A in this system given (*114*). Brown (*59*) has reported the separation and determination of vitamins A and E by paper chromatography. In this technique, vitamin A is effectively separated from tocopherols on silicone-impregnated paper strips in an acetonitrile-water system. The alcohol, acetate, and palmitate of vitamin A may also be separated from each other. Quantitative results are obtained by an automatic scanning and recording technique (*60*). The fluorescent properties of vitamin A and its esters continue to receive some attention (*7, 133*) as the basis for fluorometric procedures for this vitamin.

One of the most perplexing problems facing the pharmaceutical analyst today is that of the determination of small amounts of vitamin D in the presence of relatively large amounts of vitamin A. The very high therapeutic potency of the various forms of vitamin D necessarily imposes microchemical restrictions in the techniques which are potentially applicable. Official methods for the control of vitamin D in complex mixtures are thus necessarily still confined to the biological procedure. The cost of biological analysis and the time-consuming features of such methods are, of course, undesirable in terms of routine control methods. It would, therefore, seem likely that considerable effort would be extended toward the elucidation of a suitable chemical control procedure; relatively little in the recent literature, however, points to a potential solution to this problem.

In general, chromatographic methods of separation appear to be most likely to offer hope in this direction. Talc treated with a chloroformic solution of antimony pentachloride and dried at 100° C. has been used as an adsorbent (*63*). The technique is described and results are tabulated, indicating better than 90% recoveries. Perhaps the closest approach to a suitable analytical method is the work of Ewing *et al.* (*113*), who utilize a Superfiltrol column to remove vitamin A, carotenoids, pigments, and some sterols. A subsequent passage over an alumina column removes other interfering substances. Vitamin D is ultimately determined in the eluates by measuring the ultraviolet absorption of the solutions at 265 mμ. Concentration limits, however, are a minimum of 4000 U.S.P. units of vitamin D and not more than 75,000 units of vitamin A in the sample. The ratio of A to D should not exceed 10 to 1. A reversed phase system for the paper chromatographic separation of vitamin D and related sterols has been described by Kodicek and Ashby (*211*). In this case, the paper was impregnated with liquid paraffin. Several satisfactory solvent systems were found. Vitamin D was quantitatively estimated by location of the zone on examination in short-wave-length ultraviolet light. The zone was then cut out of the paper, and extracted with methanol and the ultraviolet absorption of the extract was measured at 265 mμ. Unfortunately, considerable interference from vitamin A was encountered.

An interesting modification of the Emmerie-Engel method for the determination of tocopherols is reported by Domart (*98*), who utilizes potassium fluoride to form a colorless complex with the excess ferric ion remaining after reduction by the vitamin E present. This procedure may have advantages in stopping the reduction reaction after a specific time. The nitrosation method for tocopherol assay has been modified to eliminate the production of interfering ethyl nitrite (*288*). The solvent used as an inert medium in this case is 1,2-dimethoxyethane instead of the previously employed ethanol.

Paper chromatography has again played an important part in separation methods for the various tocopherols. A reversed phase system has been described by Brown (*56*) for the separation of mixed tocopherols. Whatman No. 1 filter paper was coated with Vaseline and used in 75% ethanol system. Zones were identified by spraying with 2,2′-bipyridine in ethanol followed by 0.1% ferric chloride in ethanol. Brown has also reported a quantitative refinement of this separation, whereby the separated zones are cut out of the paper and extracted and quantitative measurements are made on the extract (*57*). A similar process is reported by Eggitt and Ward (*105*). A different system for the identification of as little as 1 γ of α-tocopherol is described (*156*), wherein the paper is impregnated with ferric undecylate. The solvent system is acetone-water (4 to 1). Identification is made by spraying the strips with dilute hydrochloric acid followed by potassium ferricyanide.

GENERAL

Pifer, Wollish, and Schmall have reviewed the possibilities of application of nonaqueous titration methods to substances listed in the National Formulary IX and U. S. Pharmacopoeia XIV together with some U. S. P. XV admissions. The list of compounds includes those which have been or might be titratable in nonaqueous solvents but are not otherwise suitable for volumetric determinations (*285*). Methods for the characterization and determination of alcohols, particularly aliphatic alcohols, have not been completely satisfactory in the past; as a result, considerable effort is still being expended in the improvement of existing procedures or in the development of new and more specific methods for alcohol determination. As a characterization technique, the formation of urea complexes of long-chain alcohols, fatty acids, and esters offers new derivatives, termed inclusion compounds, for identification purposes. The dissociation temperatures (temperature at which opacity first occurs when a transparent crystal of a urea complex is slowly heated) of several compounds have been shown to be reproducible; the complexes are easily prepared and the organic compound can be recovered readily after identification (*210*).

A colorimetric method for the determination of aliphatic alcohols involves reaction with ceric ammonium nitrate to form an unstable red color. The reaction conditions must be carefully controlled in order to obtain reproducible values. Sulfate ion interferes. The molecular arrangement of the alcohols investigated appears to influence the extinction values of the individual compounds (*304*). The reaction of primary alcohols and amines with *N*-halosuccinimides has been used to differentiate primary alcohols and some position isomers (*19*, *219*). Alcohol has been determined in diethyl ether simply by measuring the infrared absorption at 2.83 microns. A correction for interference from water is made after the water content is determined by Karl Fischer titration (*84*). Benzyl alcohol has been determined turbidimetrically by formation of what is believed to be a condensation polymer resulting from the action of concentrated sulfuric acid on an aqueous solution of the compound (*204*). The condensation of phenol with benzyl alcohol yields one mole of water per mole of bis(hydroxyphenyl)-methane formed as a reaction product. The amount of water produced may be measured by distillation and trapping and the water content related to the original concentration of benzyl alcohol (*247*). The Conway diffusion apparatus has been successfully adapted to the determination of ethanol in biological fluids; the center cell contains acid dichromate solution, while the ethanol diffuses from the sample in the outer cell in approximately 20 minutes. The method would appear to be usable for materials other than biological fluids, provided no other volatile reducing constituents were present (*362*).

Some general contributions to the 2,4-dinitrophenylhydrazone method for determination of aldehydes, ketones, and similar carbonyl compounds include studies on the absorption properties of the base forms of these derivatives which produce quinoidal ions (*221*). It has also been observed that 2,4-dinitrophenylhydrazine dissolves readily in 30% perchloric acid to produce a solution miscible with ethanol and Cellosolve. Conditions for differences in color of the phenylhydrazones formed are noted (*260*). A volumetric method for the estimation of formaldehyde involves reaction with a measured amount of potassium cyanide, combination of excess cyanide ion with bromine to form cyanogen bromide, reaction of the cyanogen bromide with potassium iodide to release iodine, and titration of the iodine with standard thiocyanate solution (*214*).

Jones and Riddick (*200*) have reported a specific reaction of propionaldehyde in the presence of several other aldehydes, wherein the reaction product of propionaldehyde and ninhydrin in concentrated sulfuric acid develops a reddish blue color with an absorption maximum at 595 mμ. An aeration system for separation of the aldehyde from the sample is discussed; sensitivity of the method is 1 to 3 γ. The adaptation of 1-fluoro-2,4-dinitrobenzene as a quantitative reagent for primary and secondary amines has been reported by McIntyre *et al.* (*238*); separation is accomplished by conversion of excess reagent to dinitrophenol, extraction, and ultraviolet absorption measurement of the residual dinitrophenylamine derivative ($\lambda_{max.}$ 325 to 360 mμ). As an aid in the identification of amines, the R_f values on filter paper chromatograms of a large number of these compounds in various solvents have been determined (*49*). Quaternary ammonium compounds and certain tertiary amines have been titrated with surface-active compounds such as sodium lauryl sulfate or dioctyl sodium sulfosuccinate in the presence of dilute sulfuric acid and chloroform. The indicator used was dimethylaminoazobenzene. The postulated reaction involves formation of a stable complex between the quaternary compound and the titrant; the first excess of titrant is extracted into chloroform as lauryl acid sulfate, where it reacts with the indicator to form a chloroform-soluble complex. Acids other than sulfuric, highly ionized surface-active agents, and water-soluble solvents generally make the method inoperative (*75*).

A colorimetric micromethod for quaternary ammonium compounds involving complexation with bromocresol purple has been investigated (*120*); adsorption effects on glass surfaces with which the solutions were in contact were observed to be profound. Corrective measures, including a coating technique for glassware, were discussed. Quaternary ammonium bases and related compounds have been separated by paper chromatography in an ethanol-ammonia system and in butanol–acetic acid–water. Zones were detected with phosphomolybdate–stannous chloride or with Dragendorf's reagent (*48*).

Considerable improvement in specificity of analytical methods for bile acids and pigments has been observed. A small amount of cholic acid salt, when heated with concentrated sulfuric acid, develops a blue fluorescence; after cooling and dilution with butanol, the fluorescence may be measured quantitatively. Desoxycholic acid, several cholanic acids, 7-hydroxycholesterol, and several cholestane derivatives do not interfere (*281*). Salicylaldehyde has been shown to be a more specific reagent for desoxycholic acid than furfural (*367*). Pratt and Corbitt (*294*) have employed vanillin in 85% sulfuric acid to react with desoxycholic acid to produce a chromophore with maximum absorption at 545 mμ; cholic acid and apocholic acid reaction products have absorption maxima at 465 mμ. Short-chain alcohols, acids of low molecular weight, and water inhibit the color formation.

A method of separation and identification of bile acids includes chromatography on silica gel with solvent mixtures of pentane, hexane, ether, and acetone. Infrared curves of eluate residues (methyl esters of the bile acids) in carbon disulfide are reported (*411*). Paper chromatography has been used also to separate taurocholic, glycocholic, desoxycholic, dehydrocholic, and cholic acids. The zones were identified by spraying with cold alcoholic iodine solution or by treating with phosphoric acid and observing the papergrams in ultraviolet light (*32*). Other systems for separation are propanol-ammonia-water or propanol-ethanolamine-water (*215*) and isopropyl alcohol–*n*-heptane (*342*). The latter system has been employed with paper previously impregnated with strong acetic acid solution, which probably results in some acetylation of the paper. Reversed phase chromatographic separation of bile acids has been performed satisfactorily on a column of Hyflo Supercel saturated with chloroform-heptane, with 58% aqueous methanol as the mobile phase (*30*). The R_f values for five bile pigments in four different solvent systems are given by Stich *et al.* (*358*). These are sufficient for the differentiation and identification of the pigments.

Extracts of *Cannabis sativa* show an ultraviolet absorption maximum at 280 ± 10 mμ. The $E^{1\%}_{1\,\mathrm{cm.}}$ value is approximately 225; the resin may be detected in tobacco (*36*). Small quantities of carbon disulfide and acetone may be determined by ultraviolet absorption measurement in carbon tetrachloride solution (*6*). The quantitative separation and estimation of several organic acids by paper chromatography are the subject of a report by Isherwood and Hanes (*192*). Citric acid has been asssayed by forming pentabromoacetone by conventional procedures and measuring the compound polarographically; the precision is within about ±3% (*109*). An alkali isomerization method requiring only 1 to 10 mg. of fat has been found satisfactory for the determination of polyunsaturated fatty acids. The use of a high concentration of alkali provides greater extinction values for trienoic, tetrienoic, and pentaenoic systems than have been reported previously (*177*). Microgram quantities of fatty acids have been determined by potentiometric titration of 0.005- to 0.025-microequivalent quantities; minimum solution volume is 75 μl. (*155*).

Duncan and Porteus have separated and identified the lower straight-chain fatty acids by paper chromatography in a butanol-ammonia-water system. Quantitative application of the method is discussed (*101*). Semimicro quantities of formic acid have been estimated by reaction of the sodium salt of the acid with mercuric chloride to produce mercurous chloride; the mercurous chloride then reacts with iodine and the amount of excess iodine is estimated by thiosulfate titration (*188*). Microgram quantities of lactic acid have been determined by a reaction with copper sulfate and *p*-hydroxybiphenyl in strong sulfuric acid; the method is applicable within a range of 2 to 10 γ per ml. in the presence of 10 to 60 γ per ml. of pyruvic acid (*190*).

A reaction of histamine with tetraphenylboron produces a crystalline insoluble salt suitable for the gravimetric estimation of this substance (*419*). Another gravimetric procedure for histamine involves precipitate as the nitranilate; glycine and histidine interfere (*327*). Histamine in streptomycin and dihydrostreptomycin has been estimated by formation of a Schiff base which is extracted with a benzene-butanol mixture. Ion exchange chromatography of the mixture is performed on a column of cotton acid succinate; histamine is eluted with dilute sulfuric acid and then coupled with diazotized *p*-nitroaniline. This product is extracted with methyl isobutyl ketone and the absorbance of the solution is read at 516 mμ (*203*). It has been found possible to titrate certain aromatic nitro compounds such as *m*-dinitrobenzene in nonaqueous systems; nitrobenzene, however, displays no titratable acidity. Indicators used were picric acid and 2,4,6-trinitro-*m*-cresol (*55*).

Golden (*146*) has modified the qualitative ferrous thiocyanate peroxide test to provide a quantitative estimation of the peroxide content of vegetable oils. The microdetermination of the critical mixing temperature of ethereal oils with ethylene glycol permits the quantitative determination of the main constituent in genuine ethereal oils (*118*). Several essential oils have been separated into individual constituents on "chromatoplates" coated with silicic acid in a starch binder. The solvent system used was 10 to 15% ethyl acetate in hexane; ketones and aldehydes were identified as the dinitrophenylhydrazones (*306*). Improved methods for the estimation of sesamin, sesamolin, and sesamol have been reported recently (*31*, *361*); these include chromatographic separations as well as specific color tests for individual constituents.

An interesting technique for evaluating olefinic unsaturation involves the use of ozone as a titrimetric agent; a dye indicator is used to determine the end point of the titration (*42*). The studies of Fritz and Keen (*131*) indicate that most phenols with negative substituents are sufficiently acidic to permit their titration in a dimethylformamide medium with azo violet indicator. Phenol itself and alkyl substituted phenols are weaker, requiring ethylenediamine as a solvent with *o*-nitroaniline as an indicator. The titrant is potassium methoxide in benzene-methanol. A paper chromatographic system for the separation of phenols, phenolic acids, and their derivatives is butanol saturated with aqueous sodium bicarbonate solution; the filter paper used is buffered at pH 8 (*102*). The ferric chloride test for phenols has been greatly improved by using organic solvents with pyridine as a neutralizing agent; a table lists the colors given by 78 phenols and some enols, oximes, and thiols (*350*). An ultraviolet spectrophotometric study of mixtures of eugenol and isoeugenol indicates that the two compounds may be estimated quantitatively in the presence of each other with a simple two-component procedure involving measurements of absorption ratios at 282 and 254 mμ (*385*). Columns of silicic acid impregnated with methanol-water as a stationary phase have been used to separate mixtures of *p*-hydroxybenzoate esters. The mobile phase is varied from 60–40 Skellysolve C–carbon tetrachloride through carbon tetrachloride to 10% chloroform in carbon tetrachloride to pure chloroform. The order of elution of the esters from the column is butyl first, followed by propyl, then ethyl, and finally methyl. The eluates may be examined quantitatively by ultraviolet absorption methods or by gravimetry (*182*).

Methods for the evaluation of polyvinylpyrrolidine preparations have been developed by Levy *et al.* (*230*). Methods for estimating monomer content, molecular weight range, and viscosity are included. A method for the estimation of 8-quinolinol is based on the reaction of this compound with diazobenzenesulfonic acid in buffer solutions at pH 9 to 9.5. The resulting orange to red-orange pigment is measured spectrophotometrically (*66*). Tetrachlorohydroquinone has been determined colorimetrically by reaction with benzoyl peroxide and triethylamine; phenols and chlorinated phenols do not interfere (*89*).

A scheme for the identification of stabilizing agents and thickners has been developed by Ewart and Chapman (*112*); these authors utilize reactions with calcium chloride, sodium hydroxide, barium hydroxide, and lead acetate to differentiate such materials as pectin, algin, starch, agar, various gums, cellulose modifications, and other materials. It has been observed that iodine forms complexes with aliphatic sulfides in hydrocarbon solvents; the complexes have fairly high extinctions in the 300- to 310-mμ range. This property may be used to determine the sulfide content of mixtures (*169*). A spectrophotometric method for the determination of urea utilizes a reaction with *p*-dimethylaminobenzaldehyde in dilute hydrochloric acid to produce a pigment having an absorption maximum at about 420 mμ; hydrazine and semicarbazide interfere, but methods for their removal are given (*398*). Difficulties in determining water by the Karl Fischer titration when ferric salts are present have been overcome by preliminary complexation of the ferric ion with 8-quinolinol in absolute methanol (*222*). A reagent slightly different from the Karl Fischer reagent consists of a solution of sulfur dioxide and bromine in chloroform; a visual end point in the titration of samples for water content has been found satisfactory with this reagent; however, alcohols interfere in the titration (*24*). The Karl Fischer method for moisture determination has been applied to several pharmaceutical products; the reagent was modified slightly to utilize methyl Cellosolve in place of methanol to produce a more stable reagent (*54*).

The authors wish to state again that the omission of many published articles in this particular field in no way reflects a lack of value of these reports. Rather, it indicates the inability of any one or two analysts to keep abreast of the literature reports of all the spectacular advances being made from year to year in this and in other scientific fields.

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Pesticides

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MORE papers on analytical methods for pesticides have appeared in the last 2 years than in the preceding 3 years (*121*). The major part of these methods are micromethods, primarily for spray residue in fresh processed fruits and vegetables, as well as in fresh products, and for toxicity study. This indicates increased emphasis placed on safety by industry and by state and federal governments. The following review of methods perhaps shows a greater increase in emphasis on herbicides than any other type of pesticide, in accord with the interest that has been developing.

Macromethods are available through the Methods Clearinghouse of the Association of American Pesticide Control Officials. A similar clearinghouse for micromethods should be established. Methods for the various pesticides are also included in "Condensed Data on Pesticides," first called "Pesticopoeia," the third edition of which will soon be published (*4*). Methods are presented to the Pesticide Subdivision of the AMERICAN CHEMICAL SOCIETY at each semiannual meeting. Many such methods were presented at the Milwaukee and New York meetings in 1951, and were scheduled to be published in a volume of the *Advances in Chemistry Series*.

The tolerances published in the *Federal Register* for October 20, 1954, became official on December 20, 1954, unless challenged, in conformity with the requirements of the very recent law supplementing the requirement for tolerances in the 1938 law. Some chemicals will have a zero residue tolerance; others will require no tolerances; while specific tolerances are established for others.

CHLORINATED HYDROCARBONS

Agazzi, Peters, and Brooks (*1*) discuss combustion techniques for the determination of residues of highly chlorinated pesticides. The chloride ion is determined by amperometric titration. Applied to the determination of aldrin and dieldrin, it was used at levels of 2 to 100 γ and detected 3 to 400 p.p.m. of aldrin in fat. An accuracy within ±4 γ of pesticide is indicated. Helmkamp, Gunther, Wolf, and Leonard (*54*) present a direct potentiometric method for the chloride ion applicable to residues of chlorinated insecticides in foodstuffs. They use a pH meter and a silver–silver chloride *vs.* calomel electrode system. The system is reducible with 0.02 p.p.m. of the chloride ion and appears usable up to 10,000 p.p.m. Gordon (*40*) developed a colorimetric microdiffusion method for the determination of chloride in chlorinated insecticides—an improved and more sensitive modification of the Conway method. The chloride is oxidized by permanganate and decolorizes the dye, Fast Green. Residues of 1 to 10 p.p.m. of chlorinated insecticides in biological material can be determined. The limit of detection of chlorine is about 0.1 γ or, with a Beckman spectrophotometer, about 0.02 γ of chlorine. The chlorinated hydrocarbon may be partially identified by a calculation of reaction rates. From 10 to 100 p.p.m. may be determined in oils and fats.

Mitchell (*93*) developed a method for the separation and identification of chlorinated organic pesticides by paper chromatography. In mixtures of the alpha, beta, gamma, and delta isomers of benzene hexachloride, as little as 10 γ of each isomer may be clearly separated and identified. The same author (*94*) developed a modification of this method for use with other halogen compounds, including aldrin, isodrin, dieldrin, and endrin. This test appears positive for various halogen pesticides including chlordan, DDT isomers, heptachlor, methoxychlor, and toxaphene. Moynihan and O'Colla (*100*) described the application of paper chromatography to the analyses of chlorinated organic insecticides. They have studied the separation of the isomers of benzene hexachloride, (BHC, 1,2,3,4,5,6-hexachlorocyclohexane) and of DDT and are developing a quantitative method of determination. O'Colla (*102*) described a method for the analysis of chlorinated organic insecticides by partition chromatography on paper and on cellulose columns. He separates and determines benzene hexachloride in chlordan in commercial samples.

BENZENE HEXACHLORIDE

Schechter and Hornstein (*123*) utilized the dechlorination of benzene hexachloride in developing a colorimetric method for its microdetermination. The method can be used to determine as little as 5 γ of benzene hexachloride and may be used for spray residue and pharmacological investigations. It has been used in the range up to 100 γ. Recoveries up to 100% have been obtained with the gamma and alpha isomers, but smaller recoveries with the delta and some other isomers. Recoveries of the gamma isomer added to plant, animal, and vegetable fats and oils and soils have been 95 to 100%. Chlordan appears to interfere, although other common chlorinated hydrocarbons do not show interference.

Hornstein and Sullivan (*61*) described a method for the determination of 0.1 γ of lindane (BHC) per liter in air, using the Schechter-Hornstein method (*123*), and measuring the color photometrically. Five-tenths microgram of lindane can be determined, with a precision within about ±2%. A minimum of equipment is required and the method is comparatively rapid. Hornstein (*59*) found two colorimetric methods (*107*, *123*) suitable for determination of benzene hexachloride residues, although they may not be useful for the determination of the gamma isomer in formulations and technical grades of benzene hexachloride. While the infrared method may be used for technical grades, it is costly. The partition chromatographic procedure appears to be most widely used for determination of the gamma isomer. Klein (*73*) preferred the Schechter-Hornstein method for the determination of benzene hexachloride and its beta isomer. Klein (*75*) discussed the Schechter-Hornstein colorimetric method for the determination of benzene hexachloride in foods and suggested the need for additional study. Reith (*113*) discussed a modified Schechter-Hornstein method, sensitive to 1 p.p.m., for vegetable material. Phillips (*107*) described a method for the colorimetric detection of microgram quantities of benzene hexachloride residue in foods, including peanuts and various fruits and vegetables. The method, which is readily adapted to routine quality control, utilizes extraction with aniline and color measurement with an Evelyn colorimeter. It covers a range of 1 to 140 γ of benzene hexachloride and shows high recovery in the range of about 20 to 100 γ.

Harris (*50*), who recently replaced J. J. T. Graham, an AOAC referee for 33 years, recommended certain methods and revisions and a further study of other methods for determining pesticides. These resulted from a report on benzene hexachloride by Hornstein (*60*), parathion by Giang (*38*), pyrethrins by Kelsey (*67*), rotenone by Payfer (*105*), dieldrin by McDevitt (*86*), and rodenticides by LaClair (*80*). Graham (*43*) reported on a series of pesticide chemicals. Rosin and Radan (*120*) have developed a method for the determination of lindane in commercial benzene hexachloride. They review briefly the different types of methods which have been presented for this purpose, and call attention to difficulties with commercial samples. The method gives results within ±5% of the gamma isomer, compared with polarographic determinations at levels of 10 to 30% of the gamma isomer. Willermain (*144*) applied the Thorp solubility method to the determination of the gamma isomer in technical benzene hexachloride. Hornstein (*58*) presented a method for the determination of technical benzene hexachloride in peanuts and soils, designed to eliminate interferences previously encountered. In many instances analyses can be made directly on the sample. A methylene chloride extract is used on unroasted peanuts, but on roasted peanuts a correction is required. This colorimetric method can, in general, be applied directly to soils. Streuli and Cooke (*135*) described a sensitive polarographic method for the determination of the gamma isomer of benzene hexachloride in the presence of other isomers and other chlorinated compounds. The method has been applied to pure benzene hexachloride (lindane), natural isomeric mixtures, concentrates, dusts, and other products. The gamma isomer in the range of 10 to 40% was determined. Craig and Tryon (*21*) described an isotope dilution method for the determination of the gamma isomer in technical grade benzene hexachloride, with a standard deviation of ±0.2%. The method is specific, and has been used for samples containing 1 to 50% of gamma isomer. Riemschneider, Claus, and Schneider (*116*) have developed a method for the determination of heptachlorocyclohexane in crude benzene hexachloride, by reduction with zinc and measurement of the index of refraction. Riemschneider and Stuck (*117*) describe a polarimetric method.

DDT

Mattson, Spillane, Baker, and Pearce (*89*) developed a method for the determination of DDT and the degradation product, DDE, in human fat. A modification of the Schechter-Haller method was standardized to make a differential determination of DDT and DDE in quantities totaling 5 γ and rough estimations of 2 γ. These compounds ranged from 0 to 80 p.p.m. of DDE, representing 40 to 85% of the total in human fat. DDT was not found alone. Berck (*11*) adopted the Schechter-Haller method to the microdetermination of DDT in river water and suspended solids. DDT as low as 0.003 p.p.m. in suspended solids was determined after solvent extraction and removal of interferences chromatographically. Mitchell (*97*) described the separation and identification by paper chromatography of the ortho-para and para-para isomers of DDT, rhothane [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] (DDD and TDE), and methoxychlor.

ALDRIN-DIELDRIN

O'Donnell, Neal, Weiss, Bann, DeCino, and Lau (*103*) describe methods for the determination of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydroendoexodimethanonaphthalene (aldrin) in crop materials and aldrin residues; 0.1 p.p.m. in crop materials is detectable. Aldrin is extracted, separated by adsorption chromatography, and determined by the photometric method of Danish and Lidov or by the chlorine determination procedure of Agazzi, Peters, and Brooks (*1*). Accuracy on plant extracts is a few hundredths part per million on a crop material basis. A photometric method has a high degree of specificity for aldrin. Blanks may be in the range of 0.05 p.p.m. Interferences are eliminated, although mint, for instance, was not successfully analyzed at the 0.1 p.p.m. level. Interferences of other pesticides were studied. Mitchell and Patterson (*98*) present a method for the separation and identification of as little as 20 γ of aldrin and dieldrin by paper chromatography. Mitchell (*95*) describes a paper chromatographic method for the separation and identification of the four components: aldrin, isodrin, dieldrin, and endrin. Beckman (*8*) presents a method for the determination of aldrin and dieldrinin formulations in the presence of DDT and sulfur, utilizing partition chromatography. It has 98 to 100% recovery of 0.02- to 0.2-gram quantities. Ewart, Gunther, Barkley, and Elmer (*27*) describe a micromethod for a determination of dieldrin residues. They utilized and improved a method of high precision and sensitivity, which would detect the presence of 30 γ of organic chloride or 0.1 to 0.2 p.p.m. of dieldrin.

DILAN

Mitchell (*96*) continued a series of articles on the separation and identification of organic pesticides by paper chromatography, and described the separation and identification of the components of dilan, including primarily prolan and bulan. Jones and Riddick (*65*) present a procedure for the separation of certain organic insecticides from plant and animal tissues. A procedure for the isolation of dilan from interfering biological materials was developed by the addition of extraction to the previous method. The method has been used to separate microgram and milligram quantities of DDT, lindane, parathion, chlordan, and methoxychlor from plant and animal tissue, with an accuracy within ±5%. Jones and Riddick (*64*) present a method which is specific for nitroalkanes and thus specific for dilan, which contains two nitroalkanes in a 2 to 1 ratio. The method has been used for residue on plant and animal samples and is accurate for quantities as low as 50 γ.

METHOXYCHLOR

Kunze (*71*) reported on a simplified procedure for the determination of methoxychlor in fruits and vegetables, dairy products, and biological materials, which he believes is specific for methoxychlor and sensitive to 1 p.p.m. Claborn and Beckman (*16*) describe a micromethod for the determination of methoxychlor in milk and fatty materials, as well as nonfat feeds and foodstuffs. The method is specific for methoxychlor, uses nitration by fuming nitric acid and colorimetric determination using sodium methylate. DDT and TDE in small amounts do not cause significant interferences. With quantities from 25 to 100 γ, recovery is high. Jennings and Edwards (*63*) present a method for the determination of methoxychlor on paperboard, utilizing ultraviolet spectrophotometry. The interfering substances, different from those found in foods, were eliminated. Determinations were made within a range of 0.04 to 0.14 gram per liter of cyclohexane extract, with a precision within ±5%. Recoveries of about 93% were obtained when methoxychlor in the range of 50 to 100 mg. per square foot was added to paperboard.

ORGANIC PHOSPHORUS COMPOUNDS

Schonamsgruber (*124*) described a method for the potentiometric determination of parathion or methylparathion in formulations by titration and back-titration after saponification, using a glass electrode. Zeumer and Fischer (*152*) determined parathion by reduction with zinc, diazotization, and reaction with 1-naphthol. Giang (*36*) reported on methods for parathion and recommended that the potentiometric end point technique be studied further. Wilson, Baier, Genung, and Mullowney (*145*) proposed a modified semimicromethod for the determination of parathion in citrus oils. The end point was determined spectrophotometrically.

Norris, Vail, and Averell (*101*) presented a method for the colorimetric estimation of *S*-(1,2-dicarbethoxyethyl) *O,O*-dimethyl dithiophosphate (malathion) residues. Malathion is removed by extraction, decomposed by alkali, extracted, and determined colorimetrically. Amounts between 0.25 and 2.5 mg. in 100 ml. of extract may be readily determined. The method is being adapted to the technical product and to commercial formulations and has been utilized with a large number of fruits and vegetables. It has given 60 to 100% recovery of surface residues on fruits and vegetables and 60 to 90% recovery of residues on chemical products. Metcalf (*90*) applied the cholinesterase method to colorimetric microestimation in poisoning by organic phosphate insecticides. The method is said to be rapid and precise on finger puncture quantities of blood.

Cook (*18*) reported on an enzymatic method for organic phosphate and for carbamate insecticides and (*19*) discussed enzymatic methods, presenting a method for demeton. Gardner and Heath (*35*) have presented a chromatographic method for the quantitative determination of isomers of *O,O*-diethyl ethylmercaptoethyl thiophosphate in demeton. Giang (*38*) discussed systemic insecticides, including methods for schradan, demeton, and others, and recommended further study. Hartley, Heath, Hulme, Pound, and Whittaker (*51*) described a method for the determination of schradan. David, Hartley, Heath, and Pound (*23*) presented a method for the determination of the toxic residues of schradan, which is sensitive to 1 mg. per kg. March, Metcalf, and Fukuto (*88*) discussed a paper chromatography method for use with systemic insecticides. Two separate techniques have been used in the separation and identification of the components of technical demeton, and a third method has been used for technical schradan in translocation studies with demeton and for residues in fruits, nuts, and vegetables.

FLUORINE

Remmert, Parks, Lawrence, and McBurney (*114*) describe a method for the determination of fluorine at a level of 1 to 75 p.p.m. in plant materials, primarily forage samples. They present a procedure to overcome calcination and difficulties in previous methods and indicate that the fluorine found with the AOAC method may be related to the weight of sample. Silica may interfere. Willard and Horton (*143*) present a fluorometric method for the determination of traces of fluoride, which estimates down to 0.2 mg. of fluoride with good precision. Liddell (*84*) presents a note on the colorimetric determination of small amounts of fluorine. Venkateswarlu and Rao (*139*) estimated from 30 to 120 p.p.m. of fluorine in tea. MacIntire, Jones, and Hardin (*87*) made a critical study of fluorine losses in the calcination of analytical samples of fluoride-enriched soils. Harrigan (*48*) reported on a qualitative test for fluorine in foods, and later (*49*) recommended a qualitative test for fluorine which involves quenching of the oxine fluorescence.

PYRETHRINS

Levy and Estrada (*83*) described a rapid colorimetric method for the determination concentrations of pyrethrins down to about 0.04 mg. per ml. in extracts of pyrethrum flowers. The method has also been applied to oil-based insecticides. It gives good reproducibility, unlike earlier methods. Cueto and Dale (*22*) presented a colorimetric method for the estimation of small amounts of pyrethrins, allethrin, and furethrin, which showed a sensitivity of about 200 γ and an experimental error of about 4%. The method may be used for spray residue and for the analysis of concentrates and formulations, but cannot be used for one of these pesticides in the presence of one or both of the others. As a micromethod it was used in the range of 15 to 40 mg. Schreiber and McClellan (*125*) described a micromethod for the qualitative estimation of traces of pyrethroids. Microgram quantities were determined in impregnated paper or cloth bags. Skukis, Christi, and Wachs (*130*) described a method for the spectrophotometric evaluation of pyrethrum, which is used to determine total pyrethrins in both flowers and extracts. It is rapid and reproducible (1.0%) in a range of 1 to 20%.

Hogsett, Kacy, and Johnson (*57*) developed a more precise method for the evaluation of both large and small percentages of allethrin in commercial allethrin, which may also be applicable to formulations. Konečky (*76*) reviewed methods for allethrin which are in use and under development and recommended further study of the hydrogenation and the ethylenediamine methods of determining technical allethrin. Feinstein (*28*) described a new reaction and color test for allethrin and pyrethrins. Mitchell (*99*) presented a note on recent AOAC changes in the method for pyrethrins. He believes that the cancellation of recent changes was unfortunate and should be reconsidered, and presents evidence that those changes were an improvement in the method. Kelsey (*66, 67*) reported on pyrethrins and recommended that approval of the modification of the official procedure be rescinded.

CARBAMATES

Gard and Rudd (*34*) presented a micromethod for isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) in soils and crops. They separate by extraction, hydrolysis, and steam distillation, and measure photoelectrically. Average recovery is 89%, and the lower practical limit of sensitivity is 0.05 p.p.m. of the herbicide. This method has been used with cotton seeds, vegetables, and other crops, with a precision at the 0.05 p.p.m. level of ±0.016. Lowen (*85*) presented a modified technique for the carbon disulfide evolution procedure for the determination of the active ingredient in the manzate fungicide. He also recommended the use of the Clarke, Baum, Stanley, and Hester (*17*) method with minor modifications for the determination of residues of manzate fungicide. Levitsky and Lowen (*82*) published an alternative method for obtaining the desired dispersion, based on Lowen's method (*85*). These modifications reduce the time required for analysis and give improved precision.

Fischer (*29*) described a colorimetric method for the determination of 0.5 to 10 mg. of thiuram compounds after preparation of a copper salt. Middledorf (*91*) described a method for the determination of thiocarbamates, thiosemicarbazones, and other carbamates. Shaw (*126*) studied methods for the determination of isopropyl *n*-phenyl carbamate and related compounds. Smith, Wagner, and Patterson (*133*) reviewed the literature on volumetric analytical methods for organic compounds and included a discussion of certain pesticides.

QUATERNARY AMMONIUM COMPOUNDS

Wilson (*147*) recommended a method for determining the quaternary ammonium compounds in milk and in water solutions. Furlong and Elliker (*33*) described an improved method for the determination of the concentration of quaternary ammonium compounds in water and in milk in the range of 1 to 100 p.p.m. Fogh, Rasmussen, and Skadhauge (*31*) presented a colorimetric method for the microdetermination of quaternary ammonium compounds using bromocresol purple, and spectrophotometric determination of concentrations ranging from 0 to 25 γ per liter. Wilson (*148*) reported on the determination of quaternary ammonium salts as reineckates and recommended a method for the detection of quaternary ammonium compounds in milk (*146*). He recommended a further study of the reineckate method for the determination and identification of quaternary ammonium compounds.

ARSENIC

Bartlet, Wood, and Chapman (*7*) modified the procedure of Magnuson and Watson for the determination of arsenic in fruits and vegetables. They eliminated interferences by tin, and applied the method to canned fruit and to fresh fruits and vegetables. In the range of 5 to 20 γ, the standard error was ±0.2 γ of arsenic. Following tin extraction, the standard error was ±0.4 γ. The method was used in the range of 0.1 to 2.5 p.p.m. in fresh fruits and vegetables and 0.02 to 0.50 p.p.m. in canned fruit. Evans and Bandemer (*26*) described a method for the determination of arsenic in biological materials, and in eggs and other animal tissues. From 0.01 to 1.3 γ was determined, with a recovery of approximately 90%. Almond (*2*) developed a field method for the determination of traces of arsenic in soils, using a modified Gutzeit apparatus (10 to 350 p.p.m. in the range of 1 to 40 γ). Smales and Pate (*131*) developed a method for the determination of submicrogram quantities (0.01 p.p.m.) of arsenic by radioactivation. Young (*151*) discussed the use of ceric salts in the determination of arsenic and for other analytical uses.

MERCURY

Miller and Polley (*92*) developed a method for the determination of diphenyl mercury alone or in the presence of phenyl mercuric compounds, and presented a procedure for the estimation of diethyl mercury alone or in the presence of ethyl mercuric compounds. The error of the method was not greater than 2 γ. Polley and Miller (*108*) developed an adaptation of the diphenylthiocarbazone method for the estimation of mixed phenyl and ethyl mercuric compounds. It was used to detect as low as 2 γ of phenyl mercury and to cover a range up to 4000 γ. Klein (*72*) reported on a micromethod for mercury for use with foods and biological products, and later (*74*) recommended its adoption.

HERBICIDES

Hogsett and Funk (*56*) developed a method for the determination of residue quantities of Crag herbicide 1 in agricultural crops. With 100 grams of fresh material the sensitivity limit was about 3 γ. Residues at levels of 0.1 to 5 p.p.m. were determined on various fruits and vegetables. Smith and Stone (*132*) described a colorimetric method for the determination of 2 γ of the herbicide *N*-1-naphthylphthalamic acid residues in plant tissues. It gives 80 to 100% recovery in the 5-γ range. It was used on soils as well as crops, but some soil types give excessive interference.

Young and Gortner (*150*) described a method for the microdetermination of CMU in plant tissue and colorimetrical determination of the end point. They state the method is accurate and sensitive to concentrations of 0.01 p.p.m. on 300-gram samples of fruit tissue; with modification it should be applicable to other plant tissue and to soils. Bleidner (*12*) presents a method for the determination of micro quantities of CMU by chromatography. Interferences, including those from plant material, were eliminated and the efficiency of the separation was 95 to 100% with microgram quantities. Bleidner, Baker, Levitsky, and Lowen (*13*) described a method for determining microgram quantities of 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU) residues in soils and plants. Samples were digested in concentrated alkali, hydrolyzed, separated, concentrated, extracted, and determined colorimetrically. A few parts per billion may be determined in cane sugar and a few parts per 10,000,000 in nearly all plant tissues and soils. Interferences are minimized. Freeman and Gardner (*32*) described a chromatographic separation method for the determination of four acids in formulations of the herbicide in chloromethylphenoxyacetic acid formulations (CPA).

Warshowski and Schantz (*142*) described a method for the determination of 2,4-D in soil using a countercurrent distribution method to determine quantities less than 1 mg. Stroud (*136*) discussed a method for the determination of 2,4-D. Gordon and Beroza (*41*) described a spectrophotometric method for the determination of micro quantities of 2,4-D and 2,4,5-T utilizing partition chromatography. The method is used for small quantities of 2,4-D in liquid insecticide concentrates, insecticide dusts, and alfalfa hay extracts. Determinations are within 5% of added amounts. Mixtures of 0.5 to 4.0 mg. of the two acids were separated completely, with recoveries of 96 to 100%. With experience, results should be within 2 to 3% of the correct value. Heagy (*53*) after further study of several methods recommended approval of the total chlorine method for the determination of esters of 2,4-D and 2,4,5-T or mixtures of these in liquid herbicides, utilizing the Parr bomb boric acid procedure.

Grabe (*42*) has determined 4-chloro-2-methylphenoxyacetic acid in the presence of chlorinated and unchlorinated acids by absorption in ultraviolet light. Sjoberg (*129*) has presented an infrared spectrophotometric method for the determination of 4-chloro-2-methylphenoxyacetic acid. Sorensen (*134*) has described a method for 4-chloro-2-methylphenoxyacetic acid by isotope dilution analysis. Hill (*55*) presents a method for the determination of 2-methyl-4-chlorophenoxyacetic acid in the presence of related acids using an ultraviolet spectrophotometric method.

Barrons and Hummer (*6*) studied derivatives of trichloroacetic acid and described a colorimetric method for its determination. Tibbits and Holm (*138*) present a colorimetric residue method for trichloroacetic acid in plant tissue. Quantities as low as 25 γ per gram of fresh tissue may be measured. Hummer and Barrons have utilized the procedure with plant sap and on oil extracts. The error in direct measurement is less than 0.4%.

Wood (*149*) presents a method for the colorimetric determination of maleic hydrazide residues in plant and animal tissues and some soils. It may be used for residue below 1 p.p.m. Interferences are eliminated.

RODENTICIDES

Ramsey (*111*) reported on metals, other elements, and residues in foods, recommending methods for 1080 and a further study of several other methods. Ramsey (*112*) reported on a qualitative test and a quantitative analytical method for 1080. He recommended revised qualitative and quantitative methods. Ramsey (*110*) has reported on 1080 and recommended further study. Lasco and Peri (*81*) developed a method for the determination of ANTU. Bellack and DeWitt (*10*) proposed a procedure for the analysis of zinc phosphide rodenticides.

Coon, Richter, Hein, and Krieger (*20*) discuss problems encountered in the physical determination of warfarin. They describe a modified procedure for use with mixtures which have previously given difficulty. LaClair (*79*) studied methods for the determination of warfarin, comparing results by the Eble method with bioassay, which he used as a referee method. Further study of warfarin methods is suggested.

BIOASSAY

The Boyce Thompson Institute continued the study of bioassay methods for detecting spray residues in foods and for evaluating new pesticide chemicals. Burchfield, Hilchey, and Storrs (*14*) developed a photomigration method for bioassay using mosquito larvae, based on the tendency of larvae to move away from a strong light source. Information was earlier published as a news report under the title "Analysis by Paralysis." The method has been used for the determination of spray residue, and should also be useful in studying synergism, antagonism, absorption rates, and various physical chemical processes in insect toxicology. Burchfield, Redder, Storrs, and Hilchey (*15*) developed standardized procedures for the rearing of larvae for use in insecticide bioassay, bringing together a complete description of the method partially presented in previous papers. The bioassay method secures results in an hour or less at concentrations of 0.1 to 1.0 p.p.m. Hartzell, Storrs, and Burchfield (*52*) compare chemical and bioassay methods for the determination of traces of chlordan and heptachlor in food crops. The highest average residue found was 0.047 and the lowest was 0.003 p.p.m.; most samples contained less than 0.05 p.p.m. Average results by the two methods agreed closely for chlordan, and the comparative results were fairly satisfactory, considering the very small amount of heptachlor found.

Sun and Sun (*137*) applied a microbioassay method utilizing flies to the determination of dieldrin, aldrin, lindane, isodrin, endrin, and DDT in milk. The average LD_{50} for dieldrin was found to be 0.96 p.p.m. A sensitivity of 0.1 p.p.m. was obtained. Davidow and Sabatino (*24*) discussed development of a screening test for chlorinated pesticides. This is much needed to survey a large number of food samples for pesticide residues in order to segregate contaminated samples at a minimum cost in time and money. Biological methods with flies and with mosquito larvae do not seem suitable for certain types of samples. They determined the approximate sensitivity of goldfish to DDT, lindane, heptachlor, toxaphene, aldrin, dieldrin, chlordan, methoxychlor, and dilan and suggested the use of goldfish for the rapid sorting of foods contaminated with excessive amounts of DDT, lindane, toxaphene, methoxychlor, and dilan.

OIL EMULSIONS

Riehl, Gunther, and Beier (*115*) applied the photoelectric colorimeter to the determination of oil deposit on sprayed fruit. A specific weight of a red oil-soluble dye was added to the petroleum oil. Behrens and Griffin (*9*) have presented a method of evaluating emulsifiers for formulation purposes. They evaluated the factors affecting the efficiency of emulsifiers and developed a standardized method of checking efficiency. Griffin and Behrens (*44*) describe an apparatus for the examination of emulsions involving measurement of separation.

Vonnegut and Neubauer (*140*) describe a procedure for the detection and measurement of aerosol particles, which may be used to give information regarding the concentration of particles and particle size. Gooden (*39*) reported on the physical properties of pesticide powders, and recommended collaborative studies on various methods.

MISCELLANEOUS

Kutschinski and Luce (*78*) present a colorimetric method based on the procedure of Gottlieb and Marsh, for the determination of *p*-chlorophenyl *p*-chlorobenzenesulfonate (ovotran, K-6451) in spray residues on fresh fruit. Certain interferences were eliminated. Quantities in the range of 0.05 to 0.30 mg. were determined with a recovery of 94 to 100%. The analytical procedure should be rigidly standardized. Grummitt, Marsh, and Stearns (*45*) studied the properties of [*bis*(*p*-chlorophenyl)methylcarbinol] and present a method of analysis which may be used with an error of 2% using 1-mg. samples. Kittleson (*71*) developed a colorimetric method for the determination of *N*-trichloromethylthiotetrahydrophthalimide used as a fungicide. Quantities as low as 0.05 mg. may be determined with a reasonable degree of accuracy. The method has been utilized to determine residues on fruits and vegetables. Payfer (*104*, *105*) studied modifications of methods for the determination of rotenone.

Rooney (*119*) recommended approval of the Elmore method for the determination of organic thiocyanate nitrogen in livestock and fly sprays (*118*). Fischer (*29*) has presented a procedure for the dilution of dinitrothiocyanobenzene, dinitrorhodanobenzene, trichlorodinitrobenzene, trinitrotrichlorobenzene, and tetranitrocarbazole in an acetone solution with the development of characteristic intense color. Kirchner, Miller, and Rice (*69*) present a quantitative method for the determination of biphenyl in citrus fruits and processed fruit products using chromatostrips. Added biphenyl has been determined in citrus juices in concentrations as low as 0.1 p.p.m. and in citrus peel as high as 600 p.p.m. Peters, Rounds, and Agazzi (*106*) describe methods for the determination of sulfur and halogens using combustion apparatus. A precision of 0.03% has been obtained for both. Above 3% the accuracy may be within 1%. The method is also adaptable to the determination of chlorine-containing pesticide residues on food and forage. Dible, Truog, and Berger (*25*) present a simplified procedure for the determination of 0.5 to 50 p.p.m. of boron in soils and plants.

Sinclair and Crandall (*128*) developed methods for the determination of ethylene chlorobromide and ethylene dibromide colorimetric. Results agree closely with the volumetric method. They are, however, better for the determination of very small concentrations. The methods have also been utilized for residue in orange peel and pulp. Recovery of milligram quantities is high. Sinclair and Crandall (*127*) determined ethylene dibromide in air, and attained a high recovery with 50 to 300 mg. of ethylene dibromide. Kennett (*68*) determined ethylene dibromide and ethylene chlorobromide by absorption in alcohol and determination of the liberated halogen by the Volhard thiocyanate method. The mean percentage recovery was 99.5 ± 1.3 to 1.6, when added in quantities from 1 to 30 γ.

GENERAL

Kirk and Duggan (*70*) emphasize the need for accuracy and precision in biochemical analyses and for adequate training of chemists and clinical analysts in this field. Too much emphasis has been placed on speed rather than accuracy and dependability of results. Biological variation has been overemphasized and errors in clinical laboratories have been far larger than those usually assumed, and too often such errors may invalidate the utility of the results. A referee system is suggested to improve the situation for both research and routine analytical results in this field.

Archibald (*3*) considers criteria for the use of analytical methods in clinical chemistry. He points out that in many cases the accuracy of results has been unsatisfactory. This discussion may also be of interest to research and regulatory laboratories.

Emphasis may well be placed upon the widespread misuse and overuse of the word "test." "Test" refers to a qualitative chemical procedure and not to a quantitative procedure (*122*). Authors of papers, particularly those appearing in AMERICAN CHEMICAL SOCIETY journals, should avoid misusage and eliminate the word "test" where it makes no contribution to the meaning.

Similarly, the word "strong" is frequently misused when "concentrated" is the desired meaning. Correctly used, concentrated and dilute refer to the relative quantity of a solute present, while strong and weak refer to the relative activity. The word "run" is rather generally misused. One does not "run" a quantitative analytical procedure; rather, he makes an analysis. "Assay" is also frequently misused in referring to quantitative methods of analysis.

Gunther and Blinn (*47*) discuss the basic principles for the quantitative determination of pesticide residues. They consider the basic analytical approaches to be the evaluation by direct-measurement (selective) and the isolation followed by measurement (nonselective). Even though every foodstuff containing pesticide residues must be individually investigated in selecting the final residue method, the authors feel that there is promise of systemization and standardization. They also have in press a book (*46*) on the analyses of insecticides and acaricides.

ACKNOWLEDGMENT

Thanks are expressed to C. M. Brewster, professor emeritus, for aid in searching out references.

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Scientific Paper No. 1373, Washington Agricultural Experiment Stations, Pullman, Wash.

Solid and Gaseous Fuels

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This fourth review of the analysis of solid and gaseous fuels continues former reviews (*33, 34, 54*) and includes a bibliography of references found from September 1951 through June 1954. The arrangement follows closely that of the other reviews.

SOLID FUELS

This section discusses the sampling, testing, and analysis of such fuels as coal, coke, wood, charcoal, and briquets but does not include any reference to atomic fuels.

SAMPLING

Liddell (*99*) has discussed the literature relevant to the problem of coal sampling and the general principle on which a sampling specification should be based. The ideal specification should be flexible and should provide a self-contained estimate of its accuracy in any particular case. He is of the opinion that these requirements are not met in the present British standard method, nor do they appear likely to be met while the approach to the problem is along present lines. Sampling procedure should give an accurate sample and some measure of the variation in the lot of coal sampled. The solution of this problem consists of taking duplicate samples so that the difference between the duplicates can be used to indicate accuracy. Further, the use of subsamples will give an idea of the variation within a large quantity of coal. The sampling accuracy required will depend on the purpose of the sampling. For much routine work the accuracy of each sample could be much reduced from the present accepted standards; the total weight of coal to be examined may not be substantially altered and the over-all gain would be considerable.

Emery (*44*) has presented a thorough analysis of the theory of small coal sampling. The principal factors affecting the number of evenly spaced increments to be taken from the moving consignment of coal are the mean ash content and the maximum size of the coal, but size distribution and the variation of ash content with size may affect the sampling results to a greater or less extent. It is generally conceded that, if the sampling is designed to limit the variability of the ash determined to some predetermined value, other properties of the coal, such as volatile-matter content and calorific value, will be obtained with sufficient precision. Since the general level of ash content may vary from place to place in the coal consignment, a sampling procedure for commercial application should take this factor into consideration.

Orning (*123*) has shown that the economic sampling of a coal, which is low in value per unit weight but high in tonnage produced and contains variations in moisture and ash, requires attention to maximum piece, sample weight and applicability to estimates of ash content, method of mining as related to impurities, and methods of handling affecting mixing of varying components. The compositing of alternate increments into two gross samples, to be reduced and analyzed separately, provides one method of routine control of sampling.

Bertholf (*15*) has stated that a minimum number of increments must be taken to obtain a sample of preassigned accuracy, regardless of increment weight. The principal variances include the the true variability of the coal from time to time, the random variation in increments such as number of particles, particle weight, and ash content, and variations of reduction and analysis.

Bur (*24*) has described a sampling device that consists of three lengths of 8-inch pipe which are attached to the side of the car dumper and an angle 50° from the horizontal. Two 5-inch holes are cut into each pipe in the sections projecting beyond the edge of the dumper. As the car is dumped, six increments are automatically taken from the interior of the car contents, while coal from the top of the car is excluded. As the dumper returns to the upright position, the increments drop to a hopper and thence to a sample crusher. Comparative tests have shown satisfactory agreement with belt samples and a reduction of sampling labor time effected.

Visman and Mieerman (*186*) have reviewed their method of sampling of coal and the statistical factors involved, such as deviations, and sampling sizing.

Ward (*187*) has shown that the methods for sampling and determination of moisture in coke which have been recommended by the Joint Coke Consultative Committee have a sound basis in experimental work and statistical theory.

Frei (*51*) has discussed sampling and sample preparation of solid fuels.

PROXIMATE ANALYSIS

Rodríquez Pire (*147*) has described and discussed methods of sample preparation and analysis. The sample preparation and method for determination of moisture, ash, and volatile matter are British standard methods. The determinations of fixed carbon were made according to the American Society for Testing Materials (ASTM) and its British, German, and French counter-parts. It is pointed out that an increment in ash content of coal represents a loss in value substantially greater than the carbon it replaces—e.g., in the blast furnace operation the ash in the coke is a large contributor to the sulfur and other impurities to be dealt with. The swelling of coal was measured by the British methods. Difficulty in maintaining a uniform gas flame was minimized by passing the gas through a column of pumice stone saturated with benzene. Agglutination was measured by the British tentative standard methods of Crussard and Gauzelin, giving "psammonetric curves."

Moisture. Bryson and Pickering (*23*) have given a description of the successful application of a direct Fischer reagent (*48*) titration to the estimation of moisture in bituminous coals by using a "dead stop" end point. Prior to titration, the coal is suspended in methyl alcohol for short periods, and to improve the clarity of the end point it was found desirable, although not essential, to extend this period to at least 15 minutes. The results combined the accuracy of a distillation procedure with the speed, simplicity, and convenience of the oven-drying method.

Kreulen (*91*) has modified the method of Stansfield and Gilbart (*167*) for the determination of maximum absorption of water in coal. The coal is saturated with water, then exposed in vacuo at 30° C. to a relative humidity of 97% (over saturated potassium sulfate solution). Moisture is then determined at temperatures of 100° to 400° C. The loss in weight was constant until 250° C. when a rapid increase in loss occurred, indicating decomposition of the coal. The moisture determined at 250° C. was appreciably greater than that at 105° C. It is believed that the temperature now used in the determination of adsorption water is too low, especially for high-moisture coals.

Deinum and Goedkoop (*38*) have developed a new technique for the determination of moisture in coal which eliminates pyrolysis or oxidation of the coal during the determination. In the new procedure approximately 2 grams of coal (less than 0.3 mm.) is accurately weighed in a nickel boat, which is then immediately placed in a combustion tube. The coal is heated for 30 minutes at 105° C. while oxygen-free nitrogen (36 liters per hour) carries the moisture into an adsorption tube filled with Anhydrone. Excellent checks were obtained by either weighing the Anhydrone with the moisture or weighing the residual coal sample. Heating the coal sample to 150° C. during the analysis has very little influence on the results. The oxygen content of the nitrogen can be reduced below 0.01% by first passing the nitrogen through a column of fat coal (0.2 to 0.3 mm.) heated to 300° C.

Funasaka and coworkers (*52*) have compared the moisture determination in 10 coals using Karl Fischer's reagent with the results obtained by the ordinary heating methods and found considerable coincidence of both data as well as reproducibility.

Selvig and Ode (*160*) in investigating the equilibration method for restoring partly air-dried samples of high-moisture coals to their natural bed moisture condition, have shown the advantage of reaching moisture equilibrium from the wet state; this was especially true for lignites. For practical purposes of coal classification, bituminous and subbituminous coals, even though air-dried to relatively low moisture content, can be restored to virtually their natural bed moisture condition by equilibration at 97% relative humidity and 30° C.

Goodman and coworkers (*61*) have concluded from a series of comparative tests that, since more moisture is recovered and measured by any of the distillation methods than can be determined under comparable conditions by oven-drying methods, the volumetric methods which give the most, if not all, recoverable moisture short of that derived from coal decomposition are the most accurate. The problem of air oxidation in the low-rank, high-moisture coals is eliminated by distillation methods. The coal particle is insulated and protected at all times by the liquid or vapor phases of the oil or xylene. Considering the simplicity of the method, the size of the sample taken, and the time required for one distillation, the azeotropic distillation–xylene procedure is better than the Schoch oil dehydration method.

Brown (*21*) has reviewed published data on methods for determining moisture in coals. Methods comprise thermal drying, dessication, distillation, extraction and solution, chemical, and electrical determinations. A general comparison of methods indicates that the xylene-distillation procedure is possibly the most satisfactory but is tedious, so that the less reliable oven-drying is more commonly used.

Hartmann and Bachelard (*74*) have used a method for determining moisture in Maitland coals in which the water is extracted from the finely ground coal by refluxing with alcohol, and the critical solution temperature (cryohydric point) of the mixture of the resulting alcohol-water solution is determined with an equal volume of kerosene. This method when compared with results by the xylene-distillation method gave results 0.04% high and when compared with results by the oven-drying method gave results 0.4% low.

Polyanicheva and Savatyugina (*133*) have determined moisture in peat by heating 5 grams contained in a 100-mm. Petri dish for 5 minutes with a 500-watt infrared lamp held at a distance of 10 to 11 cm. from the peat surface.

Storchak (*168*) has found, in the determination of moisture in peat by heating with an infrared lamp, that the principal factor affecting the accuracy of the determination was excessively long heating. The nonformation of a fog on a cool glass plate or mirror held 1 to 2 cm. above the sample is a reliable test for end of heating. Samples should be ground to 0.5 to 1-mm. size and heating continued usually for 3.5 to 7 minutes.

Klempt (*89*) has determined the moisture content of a moving stream of coal by two types of measuring instruments, depending on the effect of moisture in changing the capacity of a (electric) condenser. The temperature of the condenser and the size and mineral content of the coal should be maintained constant. The effects of variations in temperature, chlorine content, bulk density, size and mineral-matter content of the coal, and variations in the voltage and frequency of the alternating current supply are discussed. The Brabender apparatus uses a parallel-plate condenser, and the results obtained with this apparatus on a uniform coal stream at one coke-oven plant differed from the laboratory results an average of 0.2% over a month's time. The Lippe Hygromtester uses two condenser plates in the same plane, with a slight separation. At a different plant the results on this apparatus agreed within 0.3 to 0.5% with the laboratory monthly average.

Ash and Mineral Matter. Schuster (*157, 158*) has stated that the method for estimation of the mineral constituents in bituminous coal suggested by Thiessen (*178*) was fundamentally improved by Holthaus (*77*), who took into account the ferric oxide and sulfate content of the coal. However, when iron carbonate is present in the coal the results are too low, as recognized by Holthaus. Better results are obtained by combining this method with the coal analysis, especially the determination of carbon dioxide content. It is then unnecessary to estimate the carbonate content of the coal from the oxides formed therefrom. This simplifies the analysis, since the oxides from the carbonates can be determined along with other unchanged constituents; the calculations are also simplified.

Georgiadis (*55*) has outlined a calorimetric process for determining mineral content of coals. In his new formula

$$Q' = (Q \times 100)/(100 - ac)$$

where Q' equals heat content as measured in the bomb, Q equals true heat content, and c equals ash content. The coefficient a, newly introduced into the formula, can be determined by given experimental methods and ranges from 1.070 to 1.086. The factor ac represents the mineral content of the examined coal.

Brown and coworkers (*22*) have discussed the mineral matter of coal and stated that the best method of estimating the mineral content of coal is that of King, Maries, and Crossley (KMC) (*36*) which involves the determination in the laboratory of six quantities: ash, pyritic sulfur, carbon dioxide, sulfur in ash, sulfate in the coal, and chlorine. For laboratories with limited resources various alternative and simpler methods are in use. It is shown that the six quantities in the KMC method are interrelated; hence estimations of mineral content can be based on the determination in the laboratory of fewer than five quantities and will have good accuracy. A formula based on ash, total sulfur, and carbon dioxide has been investigated. The compounds which make up the mineral matter in coal cannot yet be fully specified, but their interrelations present a distinct pattern which no doubt has both chemical and geological significances; this aspect has not been studied in any detail. The presence of interrelations makes it impossible to derive a reliable approximate formula for the estimation of mineral matter unless great care is exercised. To find an accurate and simplified formula, some 1400 KMC analyses have been studied and the ash (A), total sulfur (S), and carbon dioxide (CO_2) in mineral matter were chosen as a basis. It is shown that the equation

$$M = 1.06\text{A} + 0.53\text{S} + 0.74\text{CO}_2 - 0.32$$

differs very little from the KMC formula.

Venter and Coppens (*185*) have discussed the significance of the mineral matter of coal under the following classifications:

1. General considerations:
   Mineral matter, intrinsic and extrinsic
   Nomenclature and chemical composition
   Changes in the course of ashing
   Changes in the course of carbonization
2. Determination of the mineral matter of coals, by:
   The direct experimental methods of Mayer
   Experimental methods of reconstruction of the different minerals (clays, sulfates, carbonates, and chlorides)
   Methods of calculation of mineral content from normal ashes (formula of King, Maries, and Crossley)
   Methods of determination of mineral content by graphic interpolation of analytical data
3. Intervention of mineral constituents in analytical procedures
   Simple cases
   Elementary analysis
   Determination of calorific value

Grassmann (*64*) has given the reasons for the importance of the determination of "true" ash content in high ash coals and preliminary results are given by using von Pezold's method (*131*).

Meldau (*108*) has outlined a method of examinations with the electron microscope by which it is possible to determine individual mineral and ash constituents from their characteristic form.

Nitatori (*119*) has described a rapid method for ash determination in coal using an electric furnace of the hot-plate type and an oxygen gas feeder. The surface of the plate is heated above 650° C. within 10 minutes and porcelain trays containing 0.5 to 1.0 gram of fuel are placed on it. After 2 or 3 minutes two trays of the same sample are covered with the oxygen feeder and the samples are burned to ash in 3 or 4 minutes.

Tomlinson and Edwards (*179*) have described a test for determining the difference in the results of ash determination when two kinds of dishes were used. The tests carried out showed that the use of smaller flangeless dishes (49 mm. in internal diameter) gave results slightly higher than those obtained with a larger flanged dish (57 mm. in internal diameter). The difference was small, between 0.01 and 0.11%, and the accuracy of the determinations was of the same order in both cases.

Mukherjee and coworkers (*116*) have studied the sorption and desorption of moisture on coals, air and nitrogen being used as moisture carriers. Static and dynamic methods gave curves similar in shape. It was concluded that: the moisture content should be established between 0 and 95% relative humidity, estimations should be carried out in nitrogen instead of air, the coal sample should be kept under water to avoid shrinkage effects, the desorption branch of the first cycle should be chosen, and sorption experiments should be carried out at temperatures below 40° C. to avoid enhanced sorption due to activation effects. The experiments showed that the water is not retained by van der Waals forces alone but a part must undergo interaction of a chemical nature with the coal.

Ash Fusion Temperature. Lees and Finch (*95*) have used a tube furnace heated by silicon carbide elements for determining fusion points of coal ashes. Consistent results were obtained with atmospheres of hydrogen and water vapor provided that a minimum flow was maintained. Its performance is compared with results obtained with Meker and Brayshaw gas fired furnaces.

Volatile Matter. Licznerski (*98*) has found that an artificial mixture of coal and mineral matter produced during gasification a larger volume of volatile matter than could be theoretically expected. For this reason it is proposed to take into account the mineral matter, not only as ballast, but also volatile matter given off by mineral matter during gasification when interpreting the results of determination of volatile matter. The formula proposed is:

$$VM = \frac{(Pex - \text{H}_2\text{O} - Pms)}{(100 - Pms - \text{H}_2\text{O})}$$

where VM = parts of volatile organic matter. Pex = parts of volatile matter given off by mineral substance, and Pms = parts of mineral substance.

Padovani (*125*) has compared the English, Belgian, and French standard methods of determining volatile matter on a number of coal samples. He found they gave data decreasing in the order given, but they are satisfactory in themselves, since differences in the various determinations in the same samples are small by each method. The Italian method (*126*) (a modification of the English) gave satisfactory and reproducible results.

In an effort to establish a normalized method for determination of volatile matter in coal, the Technical Committee for Solid Mineral Fuels (ISO TC/27) during a meeting held in March 1950 in London,England, decided to distribute a number of coal samples (13 to 40% volatile matter content) among different laboratories of Belgium, Denmark, France, England, Italy, Holland, and Sweden. On these samples the total volatile substances, humidity included, had to be determined following three different methods: Belgian (ABS 26.13), French (A.F.NOR standard MO 3-004), and English (B.S. 1016). On the basis of the results obtained it was concluded that each of the three methods was of comparable accuracy and consistency, the Belgian being the most reproducible one. The French method gives lower results than the other two, and the English gives the highest values. The differences between the results of different laboratories for the same method are of the same order of magnitude as the differences between the results of different methods in the same laboratory. The Italian results agree with the average results of other countries except for the Belgian method, where they are slightly inferior. This is explained by use of minimum required time of heating, because the Italian furnaces were inadequate for the high temperatures requested by the method. In addition to the three methods, the same determinations were carried out making use of an Italian method published by A.N.C.C. It is similar to the English one; only the dimensions and shape of the crucible are different. The crucible itself is made of platinum (instead of quartz) with a matte surface. The results are comparable to those of the English method, and the method itself is of the same degree of relative accuracy, consistency, and reproducibility as the others.

Calorific Value. Gómez Aranda and Lusia Miranda (*60*) have described the experimental determination of heating value by combustion with oxygen in a bomb. The nomenclature of present German standards is used—e.g., "heat of combustion" is the gross value (including heat of condensation of water) and "heating value" is the net figure. Methods of calculation based on elementary analysis and proximate analysis are described.

The latter is preferable because of the simplicity of the analysis. These methods are applicable only to bituminous coals of 5 to 40% volatile matter. The calculation method of Vivien and Juptner are given, but that of Goutal is considered preferable—e.g., $P = 82C + aV$, where P = heating value in calories per kilogram, C = per cent fixed carbon, V = per cent volatile matter, and a is a function of $V' = 100V/V + C$. This equation is applicable only where there is a definite relation between the quantity and heat of combustion of the volatile matter, within the limits given above. This is frequently, but not universally, the case. The method of Gunz is also given.

For the computation of the net calorific value from the experimental gross value it is necessary to know the hydrogen content, and in cases of coals containing much sulfur this must be determined directly (*14*). A modification of the method of Belcher and Spooner (*13*) is suggested for this purpose.

Mott (*115*) has shown that the difference in the corrections for nitrogen and sulfur, as recommended by the British Standards Association and by ASTM for the determination of calorific value, is only procedural.

Sunner and Bjellerup (*172*) have found that two kinds of systematic errors appear when using moving-bomb calorimeters for determining heats of combustion with substances containing halogen, or sulfur in addition to carbon, hydrogen, and oxygen: one of a specific character depending on the presence of halogen or sulfur and another of a general character which will cause erroneous results for all substances burned. The practical importance of the last type of error in methods currently used was studied by comparing results from two different independently operated moving-bomb calorimeters. The heat equivalents were determined with benzoic acid and paraffin oil for comparison. The mean values of the heat of combustion of the paraffin oil were 10,975.1 and 10,975.9 cal. per gram. The conclusion was drawn that general systematic errors do not influence the accuracy of the method used.

Wyźnikiewicz (*196*) has shown that the Goutal (*63*) method for estimation of heating value of coals depends upon the determinations of coke, water, ash, and volatile matter. The heating value is calculated from these data.

Funasaka and coworkers (*53*) have made a statistical study on calculations of the calorific value of coal.

Stumpf (*171*) has found that the use of gelatin capsules for weighing samples of solid fuel for calorific value tests requires frequent determination of the heating value of the capsules, as these vary considerably. Somewhat better results were secured with cellulose acetate, but the best results were secured with cellulose acetate-butyrate capsules. One lot of these capsules varied only from 4758 cal. per gram after 14 days' storage under atmospheric conditions to 4779 cal. per gram when stored 9 months. Another lot varied from 4768 to 4704 cal. per gram over the same period, so that an occasional check of the calorific value of the capsules should be made.

Knorre (*90*) has reviewed the empirical formulas proposed by Dulong, Mandeleev, Vondrocek, and Konovalov. A linear relation is established between the "reducing" calorific value of organic compounds—i.e., calorific value related to the total sum of the products of oxidation—and the portions of oxygen. The heat losses by the consecutive oxidation of various hydrocarbons are generalized to an average value (Ko = 3300 kcal. per kg.). This enables the use of a new generalized formula for determining the "reducing" calorific value. Evaluation of a correction factor (m) for various natural fuels gives a practical formula for determining the gross calorific value (K_v^l) of the fuel from their analysis as follows:

$$K_v^l = Ko\, m(^8/_3\, C_T + 8H_T)\,(1 - O_T/O_p)\,(1 - O_T) \text{ kcal. per kg.}$$

where C_T, H_T, O_T are the weight percentages of carbon, hydrogen, and oxygen, respectively, in the fuel; O_p is the limited amount of oxygen for the total combustion of the hydrocarbons in the fuel; and m is a correction factor for various typical fuels: pure hydrocarbon, 1.00 ± 0.03; natural gas, 1.00; petroleum products, 1.00 ± 0.03; brown and bituminous coals, 0.98 to 0.96; anthracites, from 0.94 to 0.95.

Jenne and Schwarz-Bergkampf (*79*) have developed a technical method for determining the net heating value of coal. The necessity of determining the combustion water by certain methods involves difficulties in the computation of the net heating value from the gross heating value. The existing narrowly limited stoichiometric conditions of the solid fuels as defined in the CHO triangle of Grout-Apfelbeck's presentation of the air-dried, ash-free coal (*112*) makes it possible to establish the differential values by which the higher and lower heating values are distinguished from one another. In a diagram in which the differential values are plotted against the higher heating values, the percentage of the volatile substances serves as an accurate characterization of the coal. The higher heating value of the coal can be calculated on the basis of the calorimetrically determined gross heating value (air-dried), if the ash content and hygroscopic moisture are known, so that the differential value can be read from the diagram. By subtracting this value from the gross heating value the net heating value is obtained.

Corrales Zarauza (*31*) has tested the applicability of six different formulas for calculation of the heating value from the proximate analysis of 90 different Spanish bituminous coals and compared the calculated values with actual values. The six formulas tested were those of Goutal (*62*), Nakamura (*117*), Mantel and Hülsbruch (*106*), Schuster (*158*), and Lefebvre and Georgiadis (*96*), and a modified Lefebvre and Georgiadis for Saar and Lorraine coals.

No formula was applicable to all coals, the nearest approach being that of Goutal which was useful with 84.5% of them, and the worst being the modified Lefebvre and Georgiadis formula, being useful with only 36.7%. The worst errors were four high-ash coals (24.34 to 45.85% ash). Goutal's formula gave low results for coals with high swelling index (SI) and over 36% volatile matter in the dry, ash-free coal and low values for coals of low percent volatile matter and SI below 3. The most precise results are with formulas proposed for limited groups of coals, but even with those the precision in much less than that claimed by their proponents.

ULTIMATE ANALYSIS

Carbon and Hydrogen. Banerjee (*6*) has made the following modifications in Fenton's (*46*) method for carbon and hydrogen determination in coal: Instead of a single furnace, two tube furnaces were used. The first was 14 inches long and was kept at 800°; the second, 9 inches long, was kept at 250° to 550° and contained a packing of red lead (Pb_3O_4) and lead chromate. The length of the combustion tube was increased to 32 inches. The rate of oxygen flow was reduced to 200 cc. per minute. Several layers of 90-mesh platinum-rhodium gauze were used to cover the sample, and a roll of the same gauze was put in the combustion tube adjacent to the final position of the boat. The red lead retains the oxides of nitrogen, and the gauze prevents the escape of combustible matter. The method was used for coal, coke, coal-tar, pitch, coal extract, and montan wax with results that compared favorably with standard methods. The precision for carbon was within 0.10% and hydrogen was 0.03%.

Nitrogen. Lange and Winzen (*94*) have modified a semimicro Kjeldahl method of Beet and Belcher (*10*) for the determination of nitrogen in coals and coke and obtained very good results.

A sample (0.1 gram) of the fuel is mixed with manganese dioxide (1.5 grams) and 1.5 grams of a catalyst [prepared by mixing 32 grams of potassium sulfate, 5 grams of mercury(II) sulfate, and 1 gram of selenium powder] is added. After the addition of 8 ml. of sulfuric acid the whole mixture is boiled until it clears up and no more undecomposed fuel is visible. After cooling, 100 ml. of water is added, the decomposition flask connected to a steam distillation device, and sulfide containing sodium hydroxide solution added. This solution is prepared by mixing 9 parts of a solution of sodium hydroxide (400 grams of sodium hydroxide per liter) with 1 part of a solution of sodium sulfide nonahydrate (150 grams per liter). The escaping ammonia is absorbed by an aqueous boric acid solution and titrated with sulfuric acid.

Badami and Whitaker (*5*) have used either manganese dioxide or potassium permanganate as the catalyst in Kjeldahl determinations of nitrogen in coal.

When potassium permanganate is used, 0.1 gram of coal is treated with 10 ml. of concentrated sulfuric acid for 5 minutes before any potassium permanganate is added. About 0.05 gram of potassium permanganate crystals is then added, and this addition repeated at about 0.5-minute intervals until the solution clears. The clearing may require a total of 1 gram of potassium permanganate. If somewhat larger increments of potassium permanganate are added at a time, small but usually harmless explosions occur. These are avoided by adding increments of the above size. A boiling of 20 minutes is generally adequate. About the same amount of manganese dioxide is as effective, with a shorter time for clearing. Manganese dioxide is effective with both hot or cold solutions, potassium permanganate only with hot solutions.

Radmacher and Lange (*141*) have described the methods of Kjeldahl, Mantel and Schreiber, and Dumas for nitrogen in fuels and discussed the limits of their applicability. Unterzaucher's (*183*) modification of the Dumas method was found to give high results for coal and low results for coke. By raising the temperature of the combustion of cokes to 1000° C. accurate and consistent results were obtained. When the nitrogen content of coals was determined under the same conditions as the cokes the results were still too high because pyrolytically burned methane contaminated the nitrogen and entered the azotometer. It was shown that this methane could be oxidized completely by a layer of platinum wool 40 to 50 mm. long heated to about 1000° C. and placed directly behind the combustion boat with the sample. The results found with this modified Unterzaucher method were usually in good agreement with those obtained with the Mantel and Schreiber (*107*) method. They conclude that modified Unterzaucher method always gives reliable results. The Mantel and Schreiber method must be confined to compounds of low volatility and the Kjeldahl method to be unreliable.

Zinneke (*197*) has developed the Kjeldahl method to a general method for nitrogen determinations. The substance containing nitrogen is decomposed with hot sulfuric acid and a catalyst (40 grams of selenium powder, 15 grams of copper sulfate, 70 grams of mercuric oxide, and 900 grams of anhydrous sodium sulfate). A special apparatus is described. Analytical results are given for many substances. The gas-volumetric Kjeldahl determination is also described.

Oxygen. Wildenstein (*190*) has pyrolyzed a sample of coal in a current of purified hydrogen. The effluent gas is cracked on platinized pumice stone at 900° C. Then sulfur is removed by reduction on nickel. Ammonia gas is absorbed with boric acid, water is absorbed by Anhydrone, and carbon dioxide by sodium hydroxide plus asbestos. The residual carbon monoxide is then oxidized to carbon dioxide with iodine pentoxide. The carbon dioxide is absorbed in $0.1N$ sodium hydroxide solution containing a little sodium thiosulfate which prevents discoloring effects of hypoiodous acid and hydriodic acid. This solution is then titrated.

Bastick (*9*) has studied the methods of Schütze (*155*), Unterzaucher (*182*), and Spooner (*165*) in which the sample was pyrolyzed in a current of nitrogen, and the oxygen-containing decomposition products were transformed into carbon monoxide on a carbon column at 1200° C. The best method of oxidizing the carbon monoxide to carbon dioxide was found to be by means of platinized borosilicate glass beads as a catalyst. The oxygen content of calcium sulfate and calcium carbonate was included in the determination, that of iron oxides only partly.

Blanzat and Barbé (*16*) have described a rapid method for the determination of oxygen in coal. The coal is pyrolyzed in a current of nitrogen at 1200° C. The oxygen compound formed are reduced to carbon monoxide with carbon (benzene black) at 1200° C., the carbon monoxide oxidized to carbon dioxide in the cold with iodine pentoxide on silica gel, and the carbon dioxide adsorbed in Ascarite and weighed. The method differs from others of similar type by using commercial nitrogen, a reaction tube of fritted alumina and a very efficient iodine pentoxide reagent. The method gives good results with pure compounds. In coals, sulfur compounds do not interfere but a correction is needed for the oxygen content of the ash.

Schöberl (*151*) found that, when applied to coal analysis, none of the methods for the direct determination of oxygen in organic matter gives correct results for the oxygen content of the true combustible material. The errors are quantitatively evaluated. Best results are obtained by the use of a modified differential method in which the true content of mineral matter is used instead of the ash content, and in which consideration is given to the carbonate carbon content.

Guérin and Marcel (*70*) investigated a method based on the pyrolysis of the sample and the transformation of the oxygenated compounds to water by catalytic hydrogenation. At 325° C. and with a delivery of 2 liters of hydrogen per hour the conversion of carbon monoxide and carbon dioxide to water is quantitative. A catalyst of reduced nickel with 2% thorium proved to be satisfactory for coals low in sulfur. The method is useful for coals rich in sulfur if the gas is desulfured by passing over nickel at 150° C. and blank tests are run on the nonoxygenated sulfurated compounds. These compounds yield about 2.5 mg. of water per hour; this arises from the reduction of oxides of nickel which are always present in reduced nickel. Oxides of iron, carbonates, and sulfates interfere in the determination. Silica does not.

Guérin and coworkers (*69*) have compared two methods of oxygen analysis in coal, using 11 different coals, some high in sulfur. The first method involves pyrolysis in nitrogen at 1200° C., passage of the gases through wood charcoal at 1200° C. to give carbon monoxide, subsequent oxidation to carbon dioxide with a platinum catalyst and finally gravimetric determination of the carbon dioxide. This method is preferred to the second method involving pyrolysis at 1000° C., desulfurization of the product gases, and then hydrogenation of these gases at 325° C. with a nickel catalyst activated with 2% thorium.

Georgiadis (*56*) has used an improved Spooner method to determine oxygen in coal directly by passing nitrogen (free of oxygen and dry) through a tube, 60 cm. long, 20 mm. inside diameter, heated over 21 cm. of its length to 1150° C., 6 cm. are required for the boat containing the coal sample and 16 cm. for the charcoal plug following the sample. The oxygen of the coal is converted to carbon monoxide, the gases are passed through cadmium acetate to remove hydrogen sulfide and through various agents to remove the water and carbon dioxide, the carbon monoxide is then oxidized over hopcalite catalyst, and the carbon dioxide is absorbed and determined.

Guérin and Bastick (*68*) have developed a new method for the determination of oxygen in coals by pyrolysis in vacuum and gasometric analysis of the oxygenated compounds present. The sample is heated to 1000° C. and the gaseous products are passed over a silica cracking column heated to 900° C. The carbon monoxide, carbon dioxide, and water which are formed are determined separately and the total oxygen is calculated. The ratios of these products remain constant for each type of coal and their formation can be studied as a function of temperature. The method is time consuming, but precise and valuable as a means of investigating the structure of coals.

Wnekowska and coworkers (*192*) have modified an Unterzaucher method as reported by Aluise and coworkers (*1*) for the direct determination of oxygen in organic compounds and adopted it to bituminous coals. An enlarged apparatus for larger samples and a metallic silver absorber for the removal of hydrogen sulfide were used. The results obtained by direct determinations are closer to the actual oxygen content than calculated results.

Phosphorus. Wnekowska and Czubek (*191*) have discussed two methods for the determination of total phosphorus in coal: Determination of phosphorus in the ash, and determination of phosphorus in the coal. The first method is not entirely reliable. A new method was designed in which a modified Kjeldahl apparatus is used. The following advantages for the method are claimed: Samples of more than 2 grams of coal can be used—this is important, as the phosphorus content of coal is usually low; Kjeldahl decomposition results in determination of inorganically and organically combined phosphorus; special, very simple additions to the Kjeldahl apparatus prevent escape of volatile phosphorus compounds; pyro- and metaphosphorus compounds are converted into orthophosphates; and after sulfuric acid is separated by precipitation, orthophosphates can be determined as ammonium molybdenum phosphate. A new molybdenum reagent and mixed indicator (*82*) were used. This ensured better readings of the end point in the titrations. This method was found to be 98.48% accurate (based on phosphorus content) and good reproducibility was obtained. It was found on the basis of this method that coal ashes do not contain the total amount of phosphorus that is present in a given coal sample, and therefore "ash phosphorus" and "total phosphorus" should be distinguished.

Puri and Banerjee (*137*) have used the blue color of the reduction products of phosphomolybdic acid with *p*-methylaminophenol sulfate for a colorimetric phosphorus determination in coal ashes.

A 0.25-gram sample of the ground ash (200 mesh, and a phosphorus range of 0.2 to 1.44%) is treated with a mixture of concentrated hydrochloric acid (6 ml.) and concentrated nitric acid (2 ml.) and finally evaporated to dryness. After cooling, a mixture of equal volumes of perchloric acid (70%) and concentrated nitric acid is added and heated. When fuming starts, the heating is so adjusted that only traces of perchloric acid fumes escape. When evaporation is completed, another 8 ml. of perchloric acid–nitric acid mixture is added, heated to fuming, and finally to dryness. When cool, 5 ml. of concentrated nitric acid is added and evaporated to dryness. The siliceous matter is now fixed; next is extraction with 15 ml. of sulfuric acid (50%) followed by filtration. The filtrate is diluted to a suitable volume with distilled water; 10 ml. of this solution is used for color development. Ten milliliters of distilled water and 5 ml. of sulfuric acid (10%) are added, followed by 5 ml. of a 5% sodium molybdate solution in 10% sulfuric acid. The solution is mixed well and heated in a water bath. Three milliliters of a freshly prepared aqueous solution of meta bisulfite (20%) is added and 3 ml. of a freshly prepared aqueous solution of *p*-methylaminophenol (3%). The solution is thoroughly mixed and heated in a boiling water bath with frequent stirring. After cooling, the solution is diluted and the blue color is measured in a colorimeter.

Sulfur. Siegfriedt and coworkers (*163*) have determined sulfur by a rapid microanalytical method using combustion in a small scale oxygen bomb of new design (a Parr microbomb can be used). The residue is dissolved in water, bromine is added, and the solution is boiled, made alkaline with sodium hydroxide, and filtered. The solution is neutralized with hydrochloric acid and titrated with barium chloride solution using tetrahydroxyquinone as indicator; the end point is dark orange. A magnetic stirrer and orange light filter are used.

Davies and Jackson (*37*) have compared the Eschka and Strambi (*169*) methods for total sulfur in coal and found the Eschka method gave higher results. The latter procedure involves ignition of the ash with the Eschka mixture. The sulfur in the ash had assumed a form which could not be extracted with water. When the fusion residue was dissolved in acid, sufficient sulfur was recovered to account for the difference between the Eschka and the Strambi method. In the former method the temperature is low enough that no fusion occurs, and all the sulfur can be extracted with water.

Radmacher (*139*) has found that the Eschka fusion method is best adapted for determining the total sulfur content of a coal, preferably with a Pythagoras crucible. A modified Andrews' method is used for the determination of sulfate, in which barium sulfate is precipitated by adding barium chromate solution. The excess of the precipitant is removed by adding ammonia so that the chromate ion concentration of the solution corresponds to the original sulfate. This is titrated with iron(II) sulfate solution, the end point being determined potentiometrically or by using diphenylaminesulfonic acid as indicator.

Elphick and Gerson (*43*) have developed a method for sulfur in coal with the calorimetric bomb washings. These washings are boiled, neutralized with an excess of sodium hydroxide (approximately 0.0025*N*), brought to a boil, filtered while hot, and then after cooling titrated conductometrically against barium hydroxide solution. Significant results can be obtained when the weight of sulfur determined is not less than 0.0016 gram with an 0.8-gram sample. The results are in good agreement with those obtained by the Eschka method for all coals tested.

Förch (*49*) has examined the method of Hamaker for the determination of sulfur in fuels by burning. In the method, the solution is neutralized to methyl red and the carbon dioxide is boiled off. As methyl red is generally dissolved in alcohol, it is oxidized to acetic acid and methanol which causes serious error. The tube used for burning the fuel should be rinsed at the end of the determination because sulfuric acid may condense in the cool end. The burning of organic matter creates white mists in the quartz tube. This is not sulfur trioxide but silicon dioxide hydrate.

Radmacher and Mohrhauer (*142*) have developed an indirect titrimetric sulfate determination by modifying the chromate method of Andrews (*3*) to avoid certain errors.

Heat 100 ml. of the neutral solution containing the sulfates to boiling and add dropwise 10 ml. of a reagent prepared from 30 grams of barium chromate and 10 ml. of ammonium phosphate. Boil 5 minutes and add 10 ml. of a 22.5% sodium acetate solution. Slowly add concentrated ammonium hydroxide until the solution is distinctly basic. Boil 2 minutes more and allow to cool, then filter. Wash twice with 1.5% sodium acetate solution. Titrate the chromate ion in the filtrate with standard iron(II) sulfate in presence of dilute sulfuric and phosphoric acids. Detailed directions are given for preparing the initial aqueous solution for analysis by the Eschka, nitric acid oxidation, hydrochloric acid extraction, and bomb washing methods.

Kiriyama (*87*), in order to avoid the long time required by the Eschka and bomb washing methods, attempted to determine sulfur in coals by a direct combustion method as with iron and steel samples. By preheating and burning the samples the total sulfur could be determined quickly, simply, and accurately in 15 minutes as compared with 2 or 3 days by the previous methods.

Horikawa and Sato (*78*) have applied Beet and Belcher's (*11*) rapid method for the determination of sulfur to several Japanese coals. It proved to be simple, quick, and equal in accuracy to the Eschka method. In determining noncombustible sulfur it was superior to the Eschka method.

Lissner and Göbel (*100*) have studied the Eschka method for total sulfur as used on lignites and found that during the heating of the Eschka mixture and the lignite with a burner, the lignite smoldered and volatile organic and inorganic sulfur compounds escaped. This sulfur loss has been entirely eliminated by heating the mixture uniformly and slowly in an electric muffle furnace.

One gram of air-dried and powdered lignite is mixed in a porcelain crucible with 3 grams of the Eschka mixture and slowly heated 1 to 1.5 hours to 500° C. in an electric muffle. After 30 minutes at this temperature it is raised to 750° to 800° C. and kept there for 2 hours. The sulfur is now entirely opened up and can be determined gravimetrically as barium sulfate after leaching the melt with water to which some bromine was added.

Excellent agreement was obtained, regardless of whether the sulfur content of the lignite was as high as 5% or as low as 0.72%.

Teichmann and coworkers (*176*) have proposed to reduce the time required for the determination of total sulfur in coal by the British standard specification method by: adding a few drops of agar solution to the cold suspension of the barium sulfate precipitate—it coagulates, settles down within 1 hour, and can be filtered off through a No. 30 Whatman filter; and adding 0.2 gram of potassium perchlorate to the Eschka mixture to shorten the time of heating from 4 hours to 1 hour, without loss of accuracy.

Radmacher and Mohrhauer (*143*) have reviewed critically the oxidation and reduction methods for the determination of pyritic sulfur in fuels.

In the reduction method the pyritic sulfur is reduced to hydrogen sulfide with zinc and hydrochloric acid containing some tin(II) chloride. In certain coals reduction was incomplete. Reduction is improved by finer grinding of the fuel, but this is not always satisfactory even with a particle size less than 0.006 mm. As a more powerful reducing agent, chromium powder plus hydrochloric acid [without tin(II) chloride] was used; it completely reduced pyritic sulfur at a fineness of less than 0.015 mm. and did not attack the organic sulfur. In the oxidation method, the sulfur is oxidized by nitric acid to sulfate, and this is determined gravimetrically. A modification of the method is to treat the fuel with dilute hydrochloric acid to dissolve all nonpyritic iron and boil the residue with nitric acid, whereby all iron from pyrite is brought into solution from which it can be determined by conventional methods.

Too long an oxidation of the fuel with nitric acid can give too high values for the sulfur by forming organic acids of which the barium salts are insoluble.

Treatment for 4 hours at 60° with nitric acid (specific gravity 1.12) gave satisfactory results for German coals or similar products but failed when applied to the determination of pyritic sulfur in American coals. For these, treatment with nitric acid at 20° for 72 hours gave good results. In the determination of the iron by boiling the sample with acids, it was necessary to treat the powdered sample repeatedly with hydrochloric acid in order to dissolve all nonpyritic iron and to digest repeatedly with nitric acid to dissolve all the pyritic iron. The procedures of the three methods are described in detail.

Mielecki (*111*) has given the analytical methods for determining the various forms of sulfur in coal.

Panicker and Banerjee (*127*) have determined the available sulfur in pyritic concentrates and coal by combustion.

A 0.2-gram sample is placed in a combustion boat in a current of air at 900° to 925° C. which is passed through a combustion tube at the rate of 100 to 125 cc. per minute. The products of combustion are passed through a 10-inch bed of quartz at 900° C., and then through a 6-inch bed of sulfur-free carbon (prepared by carbonizing sucrose at 900° C.) maintained at 320° C. to 340° C. The products of combustion, which contain sulfur dioxide, sulfur trioxide, and sulfuric acid along with oxides of nitrogen are reduced to sulfur dioxide and nitrogen. The exit gas is then passed through a train of U-tubes, consisting of one tube of phosphorus pentoxide, two tubes of chromium trioxide, and a guard tube of phosphorus pentoxide to retain the water lost from the chromium trioxide. The combustion requires 10 to 12 minutes, and the sweep is continued for 8 minutes longer. The amount of sulfur dioxide is determined from the gain in weight of the two chromium trioxide tubes and the guard tube.

Hartmann and Bachelard (*75*) have made comparative tests of various methods for sulfur determination in black and brown coals and spent oxides. For coals the combustion method of Pieters and Popelier (*132*) and of Hambersin (*72*) were compared with the Eschka method. Some tests were also made on the calorimetric bomb washings, which, however, gave much lower values than the Eschka method. For spent oxides the combustion methods were compared with the fusion method with sodium carbonate and potassium chlorate and some checks were made with a method using bromine in carbon tetrachloride and nitric acid. Results given by the Pieters and Popelier method for coals agreed closely with those of the standard methods and were of equal precision; for spent oxides all methods agreed sufficiently in view of the heterogeneous nature of the material. The Pieters and Popelier method was much the most rapid and has been adopted for routine use.

Fedoseev and Lagoshnaya (*45*) have determined sulfur by burning the sample in a stream of oxygen in the presence of amorphous chromium(III) oxide.

Place 0.2 to 0.8 gram of sample in one or two boats and cover with the chromium(III) oxide which is tamped down slightly. For samples of high volatile matter, the layer of chromium trioxide is made thicker. Collect the products of combustion in an evacuated (10 to 15 mm. of mercury pressure) thick-walled flask charged with 25 ml. of 0.1*N* sodium hydroxide solution and 2 to 3 ml. of 3% hydrogen peroxide. Determine the sulfur with sodium sulfite solution either volumetrically or gravimetrically.

Halogens. Seuthe (*161*) has determined chlorine in a fuel by combustion in a stream of oxygen.

The absorption apparatus contains 60 ml. of 1% hydrogen peroxide. After the combustion of 0.5 to 1.0 gram of the fuel, the tubes of the absorption apparatus are washed out with the 1% hydrogen peroxide solution back to the combustion tube. The solution is neutralized with 0.05*N* sodium hydroxide, and a mixed indicator is used. The solution is then washed into a beaker with distilled water, and 10 ml. of cold saturated mercury cyanide added. This reaction is as follows:

$$\mathrm{HgCNOH + NaCl \longrightarrow HgClCN + NaOH}$$

The solution is then titrated with 0.05*N* sulfuric acid to a violet color; 1 ml. of 0.05*N* sulfuric acid equals 0.00177 gram of chlorine or 0.00292 gram of sodium chloride.

Belcher (*12*) has determined chlorine in coals by wet combustion with sulfuric acid in the presence of potassium dichromate and silver sulfate. The liberated chlorine is absorbed in hydrogen peroxide and determined acidimetrically after treatment with Hg(OH)CN.

Das Gupta and Chakrabarti (*36*), upon the analysis of a number of a number of Indian coals for chlorine by a modified ter Meulen method (*110*), concluded that up to 35% of the chlorine is present in organic compounds rather than as inorganic chlorides.

Alkalies. Lissner and Göbel (*101*) have made the organically bound alkali soluble by base exchange with ammonium acetate, and the total extracted alkali was determined gravimetrically, after precipitation of other cations, or flame-photometrically.

Anders (*2*) has extracted a 1-gram sample of coal with ammonium acetate solution and filtered the extract into a 250-ml. volumetric flask.

One hundred milliliters is evaporated with a drop of hydrochloric acid and finally heated to a dull red heat. The residue is moistened with dilute hydrochloric acid and filtered into a 250-ml. volumetric flask. This solution is fed by compressed air into an acetylene burner and the alkali determined by flame photometry. Standard solutions of sodium and potassium are used for comparison measurements.

Logie and Rayner (*102*) have investigated the determination of alkalies in coal ashes. The classical Smith (*164*) method for alkalies is simple and suitable for adoption as a standard method finely ground coal ash. Although it is used, it is subject to certain errors and may yield low results. It was found necessary to increase the ignition temperature from 800° to 1000° C. in order to secure complete extraction of the alkalies. Details are given of a method for coal ash and another method for the direct determination of alkalies in coal or coke. In the latter method, wet oxidation with perchloric acid is used, followed by a separation of metallic ions by means of an ion-exchange column with 150- to 200-mesh Amberlite 1R-120 resin. Methods are given for the separation of sodium and potassium.

Germanium. Cluley (*27*) has determined germanium in flue dust, coal, and coke.

Mix 0.5 gram of sample with 1.5 gram of sodium carbonate and cover with 0.5 gram more of the flux. Heat the uncovered crucible to 600° for an hour. To the cold melt add 10 ml. of hot water and allow to stand on the edge of a hot plate until the melt is completely disintegrated, cool and transfer to a distillation flask, add 8 ml. of 6*N* hydrochloric acid, and carry out the distillation as described for the phenylfluorone method (*28*).

PLASTIC BEHAVIOR ON CARBONIZATION

Free Swelling Index. The application of the swelling index test as laid down in British Standards 1016/1942, the standard D720–46 (ASTM), and the French standard M11–001 are fraught with the difficulty of correctly regulating the gas flame. Rodríquez Pire and coworkers have discussed the design and action of an electric furnace for the purpose of measuring the crucible swelling index (*148*). The same type of quartz crucible is supported by a refractory cylinder, the outer surface of which is grooved and serves to support the resistance wire. This arrangement is totally enclosed in refractory brick about 1 inch thick, the bottom brick having an aperture for allowing a pyrometer to be introduced. The outer shell is made from an asbestos-magnesia mixture. The current through the spiral is 5 to 6 amperes at 120 volts and yields 950° C. The results obtained are compared with those of the gas-fired muffle.

Swartzman and Behnke (*173*) have modified the standard ASTM method for the determination of swelling index by using a standard 350-watt cone heater and standard 15-ml. platinum crucibles. Results of numerous tests show conclusively that the free-swelling index values as judged by the buttons are exactly the same as those obtained by the standard gas burner method.

Spooner (*166*) has deduced a general relation showing that the swelling power of coal is directly porportional to the amount of tar produced on thermal decomposition and inversely proportional to the amount of porous structure of the coal. This has been reduced to the general statement that swelling is proportional to (per cent of hydrogen − 4.2)/(per cent of oxygen)^2. This relation is shown to apply, and equations are deduced for swelling with fast and slow rates of heating such as the British standard swelling test, the Gray-King coke type, and the Sheffield Laboratory coking test. These formulas are then applied in conjunction with the volatile matter of the deduction of the calorific value, ultimate analysis, and other properties of coal.

Carbonization Tests. Gómez Aranda and Auría Arbuniés (*59*) have described an apparatus for low temperature distillation of coal. It consists of a hard glass tube divided by constrictions into three parts, one at the sealed end for the sample, one for dried asbestos, and a third for active carbon. The sample is heated in an electric furnace and the "light oil" and "heavy oil" are retained in the carbon and asbestos, respectively, both of which are maintained at 105° C. by an electric heater. Water and hydrogen sulfide are retained by U-tubes filled with calcium chloride and copper(II) sulfate, respectively. Gas is measured in a gasometer. Components are determined by weighing the absorbents after the apparatus is cut apart. A comparison with the Fischer retort shows a substantial agreement in all components, except that this apparatus recovers some 2% more water and somewhat less hydrogen sulfide.

Reactivity. Bohumír (*17*) has modified the Kassler (*81*) method for determining coke reactivity. By tying the leveling bulbs of the gas burets together with a pulley system so that reciprocal motion results to move the reacting carbon dioxide over the heated coke, the method was made easier to perform. A middle roller was adjusted to compensate for volume change. The procedure and calculation are described.

Weisz and Orning (*189*) have discussed the reactivity of cokes. A reactivity test developed in the Coal Research Laboratory (*124*, *159*, *162*) is based on measurement of adiabatic self-heated rates of a fuel sample in a stream of air or oxygen. Temperatures at which the solid fuel attains self-heating rates of 15° and 75° C. per minute are reported as reactivity indexes T'_{15} and T'_{75}, respectively.

Traustel (*181*) has discussed the method for determining reactivity of various cokes based upon their reaction with a mixture of carbon dioxide and carbon monoxide.

Pourbaix and coworkers (*134*) have discussed experimental arrangements for study of the reaction of carbon dioxide with coke in the temperature range of 800° to 1100° C. Some typical results are given which show the carbon monoxide to carbon dioxide ratio obtained for several cokes.

Godbert (*57*) has described a standard apparatus for the determination of the flammability of coal and mine dusts. A mixture of pulverized coal and stone dust is blown through a small vertical cylindrical furnace at a standard temperature with a stream of air. The criterion of flammability is the projection of a red flame from the botton of the furnace into the open air. The apparatus is standardized by comparison of results with those obtained in large scale tests in the Bureau of Mines.

Guérin and Adam-Gironne (*67*) have described a test procedure in which sulfur vapor flows at a constant rate over a carbonaceous sample at 800° C., and the gaseous products are analyzed. The index of thioreactivity is defined as the percentage of the sulfur transformed into carbon disulfide.

Roberts (*145*) has described two pieces of apparatus to determine the ignition temperature of solid fuels accurately with a minimum of time and expense.

Lambie (*93*) has modified the Godbert and Wheeler (*58*) flammability apparatus to determine the flammability of peat.

Olpiński and Gabryś (*121*) have used the apparatus of Swientoslawski and Roga (*174*) for the determination of the ignition temperature of solid fuels, and the effect of particle size of the fuel and the location of the thermocouple on the reproducibility of the results were observed.

Dulhunty and Harrison (*41*) have determined some of the combustion properties of coal in an electrically heated pot furnace, about 3 inches in internal diameter at the top, about 4.5 inches deep, and shaped like a crucible. A silica flue is provided, extending nearly to the bottom of the furnace; this induces air flow down the sides of the furnace onto the fuel bed and up through the flue. One thermocouple (zero thermocouple) is placed on the furnace wall above the lower end of the flue for recording the temperature of the furnace wall and of the heated air draft away from the burning fuel. Another thermocouple (combustion thermocouple) is located at the base of the furnace; this is used to record the temperature of the burning fuel during combustion. A variable transformer is used to control the furnace temperature. The furnace is adjusted to the desired temperature and a 0.6-gram portion of coal, between 18- and 32-mesh, is dropped down through the flue. Temperatures of the combustion thermocouple are recorded each minute and plotted against time. Temperature of the zero thermocouple is also plotted and used to correct the combustion thermocouple reading for furnace temperature variations. The rate of combustion appears to vary as a definite function of the carbon content or rank. It also appears to be approximately proportional to temperature between the limits used in the case of each coal, and the relation is much the same for coals of all ranks.

REPORTS

King (*84*, *85*) has reviewed the discussions and decisions of the International Standards Organization on coal analysis.

Fieldner and Gottley (*47*) have reported the research and technologic work on coal for the year 1950.

Radmacher (*140*) has reviewed the characterization, classification, and investigation of coals.

Coppens and Venter (*30*) have presented a collection of methods for analysis and tests of coal.

Morgan and Barkley (*113*) have listed world production of coal and lignite and world resources of coal, brown coal, and lignite.

Tomlinson and Hall (*180*) have discussed analytical tolerance with particular reference to coal analysis. A method for fixing tolerances is outlined and includes the procedure to be followed when the limits are exceeded and further determinations must be made.

Averitt (*4*) has tabulated the analyses of large numbers of Iowa coals and discussed the occurrence, mining methods, and sampling of the cleaned coal.

Das Gupta (*35*) has reported recent advances in analysis and testing of coal and coke.

Thévenin (*177*) has described the methods for the thermogravimetric and differential thermal analysis of coal.

Hicks (*76*) has described the recent work of the Solid Mineral Fuels Commission of the International Organization and points out some of the fundamentals on which international agreement must be reached before detailed methods and practices can be worked out.

GASEOUS FUELS

CONVENTIONAL COMPLETE ANALYSIS

Morsing (*114*) has used a gravimetric method for simultaneous determination of hydrogen sulfide, carbon dioxide, hydrogen, carbon monoxide, methane, ethane, unsaturated hydrocarbons, and nitrogen (by difference) in fuel gases. It is particularly well suited for producer gas. The accuracy is equal to that of the common volumetric methods.

Scholz (*152*) has designed an apparatus for use in blast furnace and coke-oven laboratories. It consists of two burets arranged so that the calorific value of the gas can be read directly. A mud catcher on the absorber keeps the solution clear and a potassium hydroxide trap is on the cross-over pipe. The exhausted solution can be siphoned out and the apparatus washed and refilled without dismantling.

Madley and Strickland-Constable (*105*) have designed a microapparatus for the analysis of about 20 ml. of gas. It is useful for determining carbon dioxide, carbon monoxide, nitrogen monoxide, hydrogen, nitrogen and methane.

Robinson (*146*) has used a combustion tube consisting of an electrically heated platinum spiral in a small quartz tube in a rapid, safe, single-pass combustion analysis of natural gas.

A soap-film gas analysis apparatus for rapid and complete quantitative analysis of town gas has been described (*29*).

Hakewill and Miyaji (*71*) have discussed an apparatus for volumetric gas analysis embodying elements of originality combined with desirable features from several commercial units. Analytical results are tabulated for sevaral gases analyzed with the apparatus.

Carbon Monoxide. Pawlikowski and coworkers (*129*) have described an apparatus for determination of small quantities of carbon monoxide in gaseous mixtures after selective absorption of the individual constituents of the mixture. The method is sensitive to about 0.001% by volume. Maximum carbon monoxide concentration which can be determined by this method is 3%; time required for a single determination is about 45 minutes.

Hydrogen and Methane. Nash and coworkers (*118*) have described an apparatus and procedure for the continuous determination of methane in reformed natural gas containing up to 4% methane. The methane is oxidized to carbon dioxide which is analyzed continuously according to the method by Brown and Felger (*20*) which depends on change of electric conductance of an electrolyte when it absorbs small quantities of carbon dioxide.

Oxygen. Devey (*39*) has reported an improved reagent for the absorption of oxygen in gas analysis. It consists of 10 grams of 1,2,4-triacetoxybenzene, and 15 grams of potassium hydroxide in 100 ml. of water. It absorbs 1.5 moles of oxygen per gram-mole of reagent and does not evolve carbon dioxide during the absorption. Carbon dioxide must be removed from the gases to be absorbed, however, because of the alkaline medium.

Prchlik (*135*) has determined less than 1% oxygen in illuminating gas, with an accuracy of ±4% in purest methanol containing 0.1*N* lithium chloride in which the absorption coefficient of oxygen is 7.1 times larger than in water. The polarographic diffusion current is proportional to the oxygen content, air serves as a standard. Since the solvability of oxygen is lowered as water is added to the methanol, the latter is kept over dry calcium chloride. Hydrogen sulfide in illuminating gas is determined by its effect on the oxygen wave in *N* sodium hydroxide. The oxygen wave is diminished by the simultaneous anodic wave. The gas (250 ml.) is shaken with 10 ml. of 1*N* sodium hydroxide; 5 ml. of the solution is diluted tenfold with water, and the corresponding polarogram is compared with that of a blank. Benzene in illumination gas is determined by polarographic analysis of the products of nitration. These are nitrobenzene and dinitrobenzene depending on the relative concentration of sulfuric acid and the nitric acid used. The polarograms of the unknown are compared with those of a similarly treated known air-benzene mixture.

Dahlström and Wahlund (*32*) have found an acetic acid solution of chromium carbide useful in gas analysis because it absorbs oxygen at three to four times the rate of alkaline dithionite.

Schulek and Pungor (*156*) have worked out two titrimetric methods to determine oxygen in gas analysis.

> One serves for determining oxygen content in gas samples containing above 0.1 volume %. This method is based on the absorption of oxygen by manganese oxide at 200° to 250° C. This reagent is procured by heating manganese carbonate dihydrate in a nitrogen current at 300° C. The temperature is lowered to 200° to 250° C., the gas sample is passed through the apparatus, the granulated glass (containing manganese oxide) is washed with hydrochloric acid solution containing potassium iodide and starch, and then titrated with 0.1*N* sodium thiosulfate. When analyzing gas samples with oxygen below 0.1 volume %, a 5% solution of manganese chloride tetrahydrate is treated with 1.0*N* sodium hydroxide solution containing 5% potassium iodide and four or five drops of 1% starch. The freshly precipitated manganese hydroxide is suitable for absorbing traces of oxygen in the gas sample.

Calorific Value. Wearing (*188*) has proposed a quick graphical method for determining the heating value of mixed gases.

Hartmann (*73*) has constructed a calorimeter suitable for determining the calorific value on small samples of gas (350 ml.). It is useful on gases on high calorific value (1200 B.t.u. per cubic foot) and has been used under other conditions when the Bays (*80*) calorimeter was unsuitable.

Scholz (*154*) has made an Orsat analysis (including methane and other saturated hydrocarbons by combustion) and calorific value determination on a gas mixture. Methods are given for calculating the unsaturated hydrocarbons ethylene and propylene from the analyses and calorific value and also calculating the saturated hydrocarbons ethane, propane, and butane. Methods of securing an adequate and representative gas sample are given. Examples of the application of the method to a producer gas from coal and two coke-oven gases are given.

Density. Quirk (*138*) has outlined three general methods for the determination of the specific gravity of a gas or mixtures of gases. The principle, construction, and use of the Ranarex gravitometer are discussed in detail.

Kenward and Lucas (*83*) have given a method for the accurate determination of the specific gravity of town gas by indirect weighing.

Hydrocyanic Acid. Dittmar (*40*) has suggested a new method for determination of small amounts of hydrocyanic acid in manufactured gas. The hydrocyanic acid is absorbed in sodium hydroxide, the solution treated with ammonium polysulfide and acidified. The resulting thiocyanic acid is determined colorimetrically after the addition of iron(III) nitrate solution.

Water Vapor. Roman and Hirst (*149*) have applied the Fischer reagent for the determination of very small quantities of moisture in gases. The dead-stop end point was used. A suitable glass apparatus is described.

Brickell (*18*) has determined water vapor in natural gas at high pressures by extracting the water vapor with ethylene glycol and titration of it with Karl Fischer reagent.

Taubert (*175*) has determined the water vapor in raw gases from carbonization equipment by simple cooling and condensation of the large part of the water. The exit temperature of the gas allows calculation of the additional water of saturation.

Odelskiĭ (*120*) has given the following formula for calculating the water vapor content of natural gas:

$$Y_n = A\ (P_n/P)g/\text{cubic meter}$$

where Y_n is the water-vapor content in grams per cubic meter. P_n is the vapor pressure of saturated water vapor in millimeters of mercury (which is independent of the temperature of the gas), and P is the gas pressure.

MISCELLANEOUS

Wustrow (*193*) has modified a Leuna column (*194*). The Holm fractionating column is replaced by one which eliminated the use of liquid pentane and, by shortening the time of analysis, reduced the amount of liquid nitrogen required.

Mendoza (*109*) has reviewed analyses of gaseous mixtures, covering sampling, chemical methods of analysis, micro and semimicromethods, and physical methods.

Struck (*170*) has given methods for the determination of specific gravity and density, calorific value, gas analysis (general instructions), nitrogen by Jäger volumetric method, moisture in gas, hydrogen sulfide, hydrocyanic acid, benzene, tar, and the analytical determination of low-percentage gas constituents and impurities by the Wunsch pressure apparatus.

Guérin (*65*) has reviewed modern methods of gas analysis.

Bartley (*8*) has described an infrared absorption method for the continuous analysis of gases. The apparatus is valuable in gas and coke appliance testing in gas manufacture, coke production, and for fuel economy.

Penchev and Iordanov (*130*) have improved the apparatus of Chlopin and Lukasuk (*26*) for the determination of helium, which measures the volume of the helium after absorption of the residual gas at liquid air temperature by mercury taps.

Küs (*92*) has described an analyzer and a method for the analysis of natural gas.

> The analyzer consists of a buret, two pipets containing potassium hydroxide solution and alkaline pyrogallol, respectively, and a combustion tube with a platinum filament held at a white heat. About 35 ml. of the dried gas is measured before and after scrubbing in potassium hydroxide for carbon dioxide content, and after scrubbing in alkaline pyrogallol for oxygen. Another sample is mixed with 100 ml. of oxygen, potassium hydroxide scrubbed to remove carbon dioxide, and the sample burned by passage into the combustion tube at the rate of about 10 ml. per minute. The cooled gases are measured, scrubbed with potassium hydroxide to determine the carbon dioxide formed by the combustion, and then with pyrogallol to determine the excess oxygen. If no oxygen remains, incomplete combustion is indicated and a smaller sample should be taken. The ratio of methane to ethane is calculated from the amount of carbon dioxide formed in combustion. For slow burning mixtures, copper(II) oxide at a dull red heat in a quartz tube, such as is used in the Hempel apparatus, is recommended for the combustion.

Chlenov (*25*) has given a general method for calculation of hydrocarbons in gases that is applicable to natural gas and cracked gases. The density is determined with the gas balance or with the apparatus of Schilling. Since these gases are almost exclusively hydrocarbons of the methane series, their heating value, air requirements, and composition can be approximately calculated from the density values with the aid of simple curves. The content in hydrocarbons in the cracked gases (10 to 20%) has but little effect on the results. A correction of 2% is subtracted for the hydrogen sulfide content (above 2.5%). Corrections for other constituents (carbon dioxide, oxygen, and nitrogen) are insignificant, since their concentrations are very slight.

Francis has found (*50*) that the presence of a few parts per million of sulfuric acid vapors raises the dew point of the combustion products of manufactured gas over that which would be obtained with water vapor alone. An apparatus for measuring the dew point by the increase in the electrical conductivity of a glass surface which occurs on the deposition of a liquid film of an electrolyte, such as sulfuric acid, was developed.

Dupuis (*42*) has found a methane content of about 1% in French blast-furnace and coke-oven gases, when determined by the usual method (with eudiometer). A lower content has been reported in America and England. When the method of determination is modified so that combustion takes place at 800° C. in the presence of a catalyst on a copper(II) oxide base, the methane content of only 0.1 to 0.3% is found.

Kitagawa (*88*) has reviewed methods of gas analysis by detector tubes.

Olsen (*122*) has used a Mach-Zehnder optical interferometer to measure the chemical composition and temperature of multicomponent gas mixtures.

Barlet (*7*) has used a modified Bunte buret in an exact and rapid method for the analysis of industrial gases.

Guérin (*66*) has reviewed the apparatus and techniques of gas analysis from Hempel and Winkler to the use of the mass spectrometer.

Vdovenko (*184*) has determined the hydrogen sulfide content of a gas by continuously passing a strip of lead acetate filter paper, 20 mm. wide, through a small chamber at a rate of 20 mm. per 15 minutes. A recording apparatus for determining weight increases of iron oxide sample over which gas is passed is also described.

Rosen (*150*) has used the absorption index test to predict the butane content of natural gas with a precision of 5% based upon fractional analysis. The test consists of measuring the equilibrium pressure developed when a sample of 40 volumes of a gas is mixed with 1 volume of pure octane at 760 mm. of mercury and 100° F.

Leśniewicz (*97*) has reviewed the principal types of chemical and physical gas analyzers and described their range and application in the industrial chemical field.

Sampling. Wykoff and coworkers (*195*) have described an automatic sampler of gases at high temperatures and pressures. The equipment consists of three basic units: a liquid cooled probe for removing gas samples from within the combustor, a traversing mechanism for moving the probe stepwise through the combustor at a controllable rate, and a valve mechanism and associated glass pipets for selectively collecting samples at each probe position.

Patterson (*128*) has altered an old temperature recorder controller to give a contact for approximately 4 seconds, once every 6 minutes. This impulse was used to release water (about 120 ml.) from a gas sampling chamber, thus permitting the taking of a representative 24-hour sample.

Scholz (*153*) has described a form of aspirator which makes it possible to take a truly representative compositive gas sample over a period of time by using the pressure differential over an orifice.

Pritchard and Walton (*136*) have described an apparatus for taking samples for laboratory analysis. The sample is compressed into a small cylindrical metal container by a special hand pump. Gas is delivered at low pressures by a diaphragm type pressure regulator.

Sampling problems, apparatus, and methods of analysis for oxygen, carbon dioxide, and carbon monoxide by Orsat and

Haldane apparatus have been described by the British Standards Institute (*19*).

Richford (*144*) has described a water-displacement method for collecting a series of equal increments of high pressure natural gas over a period of time for the determination of calorific value.

McGlashen and Powell (*104*) here developed an automatic device for taking a sample of high pressure gas at a uniform rate over a selected period of time. The apparatus is based on the principle that the maintenance of a constant rate of increase in gas pressure within a gas sample container from atmospheric pressure to any selected maximum pressure over any selected time period will result in a constant flow of gas into the container over the period, provided the temperature of the gas within the container is not varied. A spring chart motor is used to readjust a pressure regulator slowly.

Tar. Lunden and Cederquist (*103*) have absorbed tar, dust, and water from producer gas in a low boiling solvent (methylene chloride plus methanol) at −60° C. The dust is filtered and weighed, and the tar is determined in one part of the solvent by evaporation of the solvent and the water. Water is determined in another part of the solvent by titration with the Fischer method.

ACKNOWLEDGMENT

The author has freely used the material as abstracted by the abstractors of *Chemical Abstracts* and herewith acknowledges this and claims only the collection and presentation of the material.

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Essential Oils and Related Products

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THIS fifth review of analytical procedures for essential oils and related products follows the general outline established in the previous reviews (*56–59*). The present paper covers the literature from October 1952 to October 1954.

There is evidence of further use of infrared and ultraviolet absorption procedures, and of a marked emphasis on chromatographic separations. However, relatively few adaptations of these methods, applied specifically to essential oils, have been reported.

OFFICIAL COMPENDIA

The British Pharmacopoeia 1953 (*11*) was published and became official as of September 1, 1953. Monographs are included for the following products of interest to the essential oil industry:

	Page
Volatile bitter almond oil	26
Anise oil	46
Benzoin	78
Benzyl alcohol	79
Benzyl benzoate	80
Cade oil	100
Camphor	114
Chenopodium oil	134
Cinnamon oil	149
Citric acid	150
Clove oil	153
Colophony	165
Coriander oil	168
Dibutyl phthalate	182
Dill oil	194
Dimethyl phthalate	197
Ethyl oleate	227
Eucalyptol	227
Eucalyptus oil	228
Glycerol	248
Lavender oil	301
Lemon oil	304
Menthol	323
Nutmeg oil	368
Oleic acid	375
Peppermint oil	406
Rosemary oil	474
Saccharin	475
Saccharin sodium	476
Santonin	479
Prepared storax	519
Tar	558
Terpineol	560
Thymol	571
Balsam of Tolu	574
Turpentine oil	590
Vanillin	597

Appendix IV, page 702, gives detailed procedures for the determination of physical properties; Appendix X, page 754, for the determination of chemical properties.

According to what little information is available, it appears that a new edition, the seventh, of the Russian Pharmacopoeia (*104*) was published in 1952.

NEW TEXTS AND PUBLICATIONS

Volume III of "Colorimetric Methods of Analysis" by Snell and Snell (*117*) was published in 1953. This volume describes colorimetric methods as applied to organic compounds; Volume IV, as yet unpublished, will conclude the series.

A second and enlarged edition of Siggia's well-known work, "Quantitative Organic Analysis via Functional Groups" (*113*), appeared in 1954.

A select and thoroughly adequate listing of texts, journals, compendia, and reports dealing with the literature of the field of essential oils was compiled by Leidy (*75*).

An excellent publication by the Syndicat National des Fabricants et Importateurs d'Huiles Essentielles et Produits Aromatiques Naturels of Grasse, France (*124*), was released. It was prepared under the direction of Igolen, Crabalona, Daumas, Benezet, and Teisseire. This volume describes methods of analysis and gives analytical constants for commercial essential oils. This represents a praiseworthy attempt to maintain the quality of commercial oils.

The British Standards Institute (*12*) described methods of testing essential oils, and gave procedures for the determination of physical and chemical properties.

The Scientific Committee of the Essential Oil Association of the United States (*41*) published specifications and standards for the following:

Oil Ocotea Cymbarum	No. 62
Oil guaiac wood	No. 63
Oil Amyris	No. 64
Oil balsam of Peru	No. 65
Oil balsam of Tolu	No. 66
Oil opoponax	No. 67
Oil olibanum	No. 68
Camphor oil 1070	No. 69
Oil linaloe wood	No. 70
Oil sage, Spanish	No. 71
Oil pennyroyal	No. 72
Oil pimento leaf	No. 73
Isobornyl acetate	No. 74
Oil cananga	No. 75
Oil sweet marjoram	No. 76
Oil clary sage	No. 77

Revisions for existing specifications of aromatic chemicals are given in No. R–1; for essential oils, in R-2. The EOA cassia flask (previously described in EOA Apparatus 1) is now available as a stock item from Kimble Glass under the number 28068. This is a flask of very convenient size for the determination of aldehydes and ketones as well as phenols.

A collaborative study of the reproducibility and application of infrared absorption techniques has been inaugurated by the Scientific Committee of this association. No published findings have appeared as yet.

Several new journals of interest to the essential oil field have been issued: *Analytical Abstracts* (*2*), published monthly for the Society for Analytical Chemistry (England); the *Journal of Agricultural and Food Chemistry* (*68*); and *Conserve e derivati agrumari* (*Palermo*) (*24*). None of these journals deals exclusively with essential oils, but they frequently carry articles of interest.

Dawson and Harris (*30*) prepared a bulletin on sensory methods for measuring differences in food quality, which includes a selected bibliography of some 300 papers.

ANALYTICAL PROCEDURES FROM SCIENTIFIC AND TECHNICAL LITERATURE

Acids. Methods employing circular paper chromatography were described for the separation and identification of certain organic acids by Giri, Murthy, and Rao (*54*) and by Airan, Joshi, Barnabas, and Master (*1*). Paper chromatography, using an ascending method, was used by Van Duuren for the determination of some nonvolatile acids (*129*). Using a paper chromatographic technique, Jones, Dowling, and Skraba (*67*) reported R_f values for a number of common acids in several solvent combinations. Duncan and Porteous (*34*) used paper chromatography for the identification and determination of lower straight-chain fatty acids. Burton (*18*) described the separation of volatile acids by paper chromatography. Micheel and Schweppe (*81*) used a cellulose acetate paper for the separation of the higher fatty acids; they also reported on a separation of isomeric phthalic acids by this same technique. Lindqvist and Storgärds (*76*) made a comprehensive study of several variables encountered in the separation of volatile fatty acids by paper chromatography, and recommended a detailed procedure, based on this study. Nijkamp (*91*) described a simple chromatographic method for the determination of C_{10} to C_{20} saturated straight-chain fatty acids, using a long column packed with silica gel.

Kawamura, Momoki, and Suzuki (*70*) reported a novel micromethod for the coulometric titration of salicylic acid.

For the potentiometric titration of substituted fatty acid, Radell and Donahue (*97*) recommended as solvent a mixture of benzene and methanol containing lithium chloride; as a titrant, a $0.1N$ solution of sodium methoxide.

Alcohols and Phenols. Ott (*95*) investigated a method using a mixture of pyridine and acetic anhydride for the determination of tertiary alcohols in essential oils; results were found to be reproducible but slightly lower than the values obtained by the classical Glichitch method. For primary alcohols, Petrova (*96*) employed as the acetylating agent a mixture of acetic acid (?) and pyridine in the ratio 1 to 3; other minor modifications of the method of Verley and Bolsing were suggested.

Brown and Hafliger (*14*) described a convenient technique for carrying out micro-Zerewitinoff determinations; several modifications were prepared to give consistent and reproducible results.

Rao, Shah, and Pansare (*98*) used the lithium aluminum hydride method (originally proposed by Higuchi) for the microdeterminations of active hydrogen in organic compounds; they described an apparatus and gave a detailed procedure.

For the separation and identification of normal aliphatic alcohols and methyl carbinols, Van Duin (*128*) used as reagent a 4-dialkylamino-3,5-dinitrobenzoyl chloride; this gave colored esters which were separated by partition chromatography.

Yarborough (*134*) reported on the mass spectrophotometric analysis of alcohols containing six and seven carbon atoms; of the mixtures studied, analytical results were obtained agreeing within ±5% of the true values.

Chang (*23*) compared the separation of menthol from essential oils by fractional distillation with that by chromatographic adsorption; results obtained by using a column packed with activated carbon (100- to 200-mesh) were good.

Rosenthaler and Vegezzi (*101*) studied the influence of sulfuric acid concentration on the color which developed when aliphatic alcohols were treated with aromatic aldehydes—e.g., vanillin, *p*-hydroxybenzaldehyde, salicylaldehyde, and ethylvanillin. Such color reactions must be interpreted with caution when applied to mixtures as complex as essential oils.

Böhme and Opfer (*9*) found that pseudosaccharin chloride gave crystalline products with some 25 glycol and glycol derivatives which they studied; this reagent had been suggested previously by Meadoe and Reid (*80*) for the identification of alcohols.

A chromatographic separation of normal aliphatic alcohols having one to five carbon atoms was reported by Dal Nogare (*28*); columns of silicic acid and of a mixture of silicic acid and Celite were used. Such columns were not adequate for the separation of the iso form from mixtures with the normal alcohols. The same technique was suitable for the separation of glycols having two to four carbon atoms.

Bergner and Sperlich (*7*) recommended chromatography for the determination of glycols in foods, cosmetics, and similar mixtures.

Griffin (*55*) reported on the determination of glycerol and propylene glycol in desiccated coconut by a distillation technique using Decalin and a Dean and Stark type of receiver. The high boiling point of Decalin should make this compound useful in determinations in the essential oil industry.

Mathers and Pro (*79*) described an elaborate method for the determination of propylene glycol and glycerol in foods and medicinals.

Fritz and Keen (*50*) suggested a technique for direct titration of phenols in a nonaqueous medium. Dimethylformamide was studied as a solvent with azo violet as indicator. For alkyl-substituted phenols, ethylenediamine was recommended as solvent with *o*-nitroaniline as indicator. If such a method could be adapted for use with essential oils, it would prove very useful in special cases.

Fischer and Hall (*47*) studied critically the method of the United States Pharmacopoeia for the determination of eugenol in clove oil. They recommended that the flask be thoroughly shaken at least three times during the heating period and be permitted to stand for 20 hours before the reading is taken; a modified cassia flask was also suggested. Analysts experienced in the analysis of clove oil have found that shaking is necessary to assure adequate mixing of the oil and alkali for complete saponification of the eugenyl acetate. The Essential Oil Association flask, now available from Kimble Glass, gives more accurate results than the flask proposed by Fischer and Hall.

Esters. For the determination of the saponification value of low boiling esters Davis (*29*) suggested the use of a balloon attached to the side arm of the reflux tube; this results in a closed (but expandable) system and prevents loss of the volatile esters.

Fischer and Hall (*45*) reported on the assay methods of the United States Pharmacopoeia for esters in volatile oils, and proposed modifications.

Dullaghan and Nord (*33*) described an iodometric procedure for the determination of glycidic esters. The method is based on Darzen's contention that glycidic esters react quantitatively with hydrogen iodide to yield acyclic esters and free iodine.

For ascertaining the cinnamein content of Tolu balsam, Rosenthaler (*102*) suggested triturating a 1-gram sample with 5 grams of sand before extracting with ether; this assures complete extraction.

For the separation of methyl esters of fatty acids having 12 to 22 carbons atoms, Cropper and Heywood (*26*) used a vapor phase chromatographic technique. Columns packed with equal parts of Celite and high vacuum silicone grease were employed. Nakabayashi (*86*) determined R_f values for several coumarins using paper chromatography.

To detect benzoates and hydroxybenzoates in foods and pharmaceuticals, Gakenheimer (*51*) used the diazotization technique of Edwards *et al.* (*37*).

Aldehydes and Ketones. Montes and Grandolini (*84*) studied the hydroxylamine method of Bryant and Smith for aldehydes and ketones, and applied it to 140 compounds. Feuell and Skellon (*43*) proposed the addition of sodium acetate (or ammonium acetate) to the blank to permit a more accurate color match; this proved advantageous in the determination of carbonyl compounds when hydroxylamine or semicarbazide was used.

For the determination of aldehydes in the presence of acids, ketones, acetals, and vinyl ethers, Siggia and Segal (*114*) developed a method using a modified Tollens reagent; the excess ionic silver was titrated potentiometrically with potassium iodide solution. Such a method for determining aldehydes in the presence of acetals and aldehydes in the presence of ketones should prove of much value to the essential oil industry.

Fischer and Hall (*46*) studied the official method of the United

States Pharmacopoeia for the assay of cassia oil and suggested several much needed modifications. The use of the new Essential Oil Association cassia flask would prove more advantageous than the use of the special flask proposed by the authors. A novel method for the determination of aldehydes using bisulfite was proposed by Salomaa (*106*). A measured amount of a solution of sodium bisulfite containing 5 to 10% of ethyl alcohol was made to react with the aldehyde and the excess bisulfite titrated with a $0.1N$ iodine solution. To prevent oxidation, the reaction mixture must be covered with a layer of toluene.

For the determination of aldehydes and ketones with phenylhydrazine, Terent'ev and Zabrodina (*125*) described the use of a solution of the hydrochloride in pyridine; the excess reagent was titrated with a 0.1 to $0.2N$ copper acetate solution.

Nivoli (*92*) described the preparation of alumina columns for the separation of vanillin and coumarin from toluene solutions. Sjöström (*116*) utilized an ion exchange column (Amberlite IRA 400) for the separation of ketones as the bisulfite addition compound. Newcombe and Reid (*90*) suggested treatment of filter paper with sodium bisulfite to separate carbonyl compounds on the basis of degree of reactivity.

Lagneau (*74*) used paper chromatography in a study of vanilla extracts to separate vanillin, ethylvanillin, and heliotropin; R_f values were reported. Sýkora and Procházka (*123*) identified carbonyl compounds by a paper chromatographic separation of the 2,4-dinitrophenylhydrazones. Kramer and Van Duin (*72*) separated and identified the 2,4-dinitrophenylhydrazones of normal aliphatic aldehydes and ketones having 18 carbon atoms or less by partition chromatography using silica gel.

A micromethod for the determination of carbonyl compounds using nitro-substituted phenylhydrazines was reported by Schöniger (*109*); the excess reagent was reduced with a titanium trichloride solution and the excess of this latter solution in turn determined by titration. The procedure appears too complex for use as a routine method.

Siggia and Segal (*115*) proposed the use of primary amines for the determination of aldehydes. An excess of a $2M$ solution of lauryl amine was used, and was determined potentiometrically by titration with a salicylic acid solution. Methyl ketones cause some interference, but other ketones in moderate concentrations have little or no effect.

Onoe (*94*) studied factors affecting results of chromatographic separation of 2,4-dinitrophenylhydrazones of alphatic compounds, using the glass chromatostrip technique of Kirchner and Miller. R_f values were sensitive to variations in the moisture content of the strip, so that the extent of desiccation must be controlled in preparing such chromatostrips. Isomeric forms of the individual aliphatic aldehydes could not be separated from the normal compounds.

Howard and Tatchell (*63*) separated milligram quantities of the 2,4-dinitrophenylhydrazones using a reverse phase partition chromatographic column.

Souchay and Graizon (*119*) reported a polarographic technique for the determination of aldehydes and ketones using semicarbazide. Hasselbach (*61*) described the polarographic determination of citral and citronellal in essential oils.

For determination of α-diketones, Modiano and Pariaud (*83*) used bromine in the presence of mercuric sulfide. Englis, Fisch, and Bash (*38*) measured absorption of dimethylglyoxime at the peak near 226 mμ for determining as little as 0 to 10 p.p.m. of diacetyl; results compare well with the gravimetric method in which the nickel complex of dimethylglyoxime is precipitated and weighed.

Ross (*103*) triturated the 2,4-dinitrophenylhydrazones of ketones with mineral oil and determined infrared absorption spectra in the range of 6 to 15 microns and gave spectra for many such derivatives. Naves and Lecomte (*89*) reported the infrared absorption spectra of ionones, irones, and several of their derivatives.

Karrer and Blass (*69*) described a color reaction for α-ionone and 5-oxy-α-ionone. The addition of a few drops of 20% sodium hydroxide solution gives a red color showing a maximum at 510 mμ; the color gradually fades, however. As β-ionone does not show this color reaction, these authors suggested the test could be used to ascertain the presence (or absence) of α-ionone in β-ionone preparations.

Ensminger (*39*) reported the ultraviolet absorption spectra of vanillin, ethylvanillin, and coumarin in alkaline and acidic solutions for the determination of these compounds in imitation vanilla extracts. Ensminger (*40*) reported on a collaborative study of the official gravimetric method of the Association of Official Agricultural Chemists for the determination of vanillin and coumarin in the vanilla flavors; low and variable recoveries were found.

For the detection of ethylvanillin in imitation vanilla extracts, Janovsky and Filandro (*65*) suggested the use of a color reaction with a 1% freshly prepared solution of *p*-aminophenol in alcohol.

Broderick (*13*) questioned the value of the lead number in the evaluation of vanilla extracts; he concluded that as an index of quality, the lead number is useless. However, as an index of purity the lead number cannot be ignored.

Cartwright (*21*) reported the solubility and volatility of vanilla-like synthetics—propenyl guaethol, bourbonal, vanillin, and coumarin.

Castiglioni and Bionda (*22*) detected heliotropin in a mixture with vanillin by a condensation with cyclohexanone; separation of the precipitate and subsequent treatment with sulfuric acid yielded a violet color characteristic of piperonylidenecyclohexanone.

As a reagent for the identification of aromatic aldehydes in essential oils, Klosa (*71*) proposed the use of 4-hydroxycoumarin. He gave melting points for derivatives of several aromatic aldehydes; aliphatic aldehydes other than formaldehyde and acetaldehyde do not react.

For the determination of small amounts of acetone Etienne (*42*) proposed spectrophotometric measurement of the condensation product with furfural.

Costa and Cardosa do Vale (*25*) applied the method of Garratt for the detection of furfural to essential oils.

For the determination of *tert*-butyldimethylacetophenone and musk ketone, Bogdanov (*8*) developed a gravimetric method based on the condensation of those compounds with anisaldehyde in the presence of a 40% sodium hydroxide solution.

Johnston (*66*) offered a modification of the hydroxylamine method for the determination of aldehydes; *tert*-butyl alcohol is used as a solvent to prepare the solution of hydroxylamine. The use of a tertiary alcohol precludes the possibility of acetal formation. A pH meter is used to establish the end point. These modifications eliminate the two basic problems in the use of an oximation method for the determination of aldehydes. This procedure should be thoroughly investigated by other laboratories, as it offers much promise.

Terpenes. A method employing paper chromatography was described by Schenck and Frömming (*107*) for the determination of azulene in the essential oil of chamomile. For the determination of azulene in chamomile flowers, Fischer and Resch (*48*) suggested a simple method: distillation of the oil with steam and subsequent measurement with a photoelectric colorimeter.

Xavier, Verghese, and Yeddanapalli (*133*) estimated *p*-cymene by oxidation to terephthalic acid; they reported that rapid oxidation gave better results than slow oxidation.

Sutherland (*122*) published a refractive index–density (*n-d*) chart for 25 naturally occurring terpenes and described its use.

Nitrogen-Containing Products. Freeman (*49*) described three methods for the determination of Schiff bases by titration in nonaqueous media.

For the determination of hydrogen cyanide in drugs, Torres (*126*) proposed a simple distillation technique wherein the hydrogen cyanide is distilled through a condenser, the tip of which is immersed in a 1% solution of sodium hydroxide; the resulting sodium cyanide is determined by titration with a solution of silver nitrate.

Wojahn (*132*) described a novel method for the determination of isothiocyanates by oxidation with hypoiodite. As a routine test, the method of the eighth edition of the National Formulary appears to be more useful.

Schultz, Gmelin, and Keller (*112*) isolated mustard oil glucosides with the aid of anion exchange resins. Schultz and Gmelin (*110*) also investigated a method using paper chromatography for such isolation. For the quantitative estimation of mustard oil glucosides these authors (*111*) proposed a color reaction with an anthrone reagent; the color intensity was measured photometrically. Interfering substances must first be removed, preferably by paper (or ion exchange) chromatography.

McKenzie and Wallace (*77*) made a critical study of the Kjeldahl method for the determination of nitrogen; digestion conditions, temperature, catalysts, and oxidizing agents were investigated.

Peroxides. Beckett and Dombrow (*4*) used a polarographic method for the determination of ascaridole in chenopodium oil and in castor oil solutions of chenopodium oil. In a subsequent paper, Beckett and Jolliffe (*5*) compared the polarographic method with the iodometric method of the British Pharmacopoeia. They criticized the official method because of the use of an incorrect factor and because the amount of iodine released is not directly proportional to the weight of ascaridole. In spite of the obvious deficiencies of the official method, it has received acceptance because of the lack of a more adequate procedure that can be used as a routine test for the commercial evaluation of this oil.

Maruyama (*78*) investigated the polarographic determination of ascaridole and reported an accuracy within ±1%. Garratt and Phipers (*52*) assayed ascaridole in castor oil solutions of chenopodium oil by a distillation with ethylene glycol, separation of the oil from the distillate, and final determination of ascaridole by the official method of the British Pharmacopoeia.

For the determination of organic peroxides, Sully (*121*) suggested a modified iodometric determination.

Torres and de Diego (*127*) proposed the introduction of the "oxidation factor" as a chemical property for the evaluation of essential oils. The oxidation factor was defined by these authors as the number of milligrams of essential oil oxidized by 1.0 ml. of a 0.1*N* dichromate solution; the value increases with age.

Determination of Essential Oil Content. Schirm (*108*) made a study of distillation methods for the determination of the essential oil content in drugs. He reported that the addition of sodium chloride decreased the water solubility of certain components of the oil and shortened the time required for distillation. The use of a measured amount of xylene was also recommended to lessen the tendency of the oil to dissolve in the aqueous distillate and to aid in the washing down of the condenser.

Stahl (*120*) described a new apparatus using continuous steam distillation for the determination of small amounts of essential oil. The introduction of 0.5 ml. of pentane in the receiver was recommended. Brückner (*15*) developed a microapparatus for estimating the content of essential oil in a 2-gram sample of peppermint leaves. The receiver is a capillary graduated in 0.001-ml. units. Bournot (*10*) compared this apparatus with others and reported satisfactory results especially if only a small sample of the botanical was available.

An oxidation method was proposed by Hadorn (*60*) for the determination of essential oil in spices. This method cannot be recommended as a general procedure for the essential oil industry.

Carson (*19*) reported a collaborative study of the official method of the Association of Official Agricultural Chemists; wide variations were noted in the results obtained for nutmegs and allspice (Pimenta berries). The use of a special receiver, applicable for oils heavier than water as well as for oils lighter than water, was investigated; in a subsequent paper, Carson (*20*) reported that this receiver proved unsuccessful for allspice.

Determination of Water Content. Duvall and McBay (*35*) applied the Karl Fischer method successfully to some 34 official drugs. The use of methylene blue (in absolute alcohol or pyridene) was proposed by Fischer (*44*) as an aid in the visual determination of the end point.

Belcher and West (*6*) proposed a new reagent for the titration of water; it is similar to the Karl Fischer reagent but uses bromine to replace iodine. Results were reported to be more nearly stoichiometric and the end point to be more easily defined visually. For potentiometric titration, it proved as satisfactory as the original Karl Fischer reagent.

Aubry and Monnier (*3*) suggested an elaborate method for the determination of small amounts of water in solvents; a saturated solution of lithium bromide in anhydrous ether is added and the hydrate is thus precipitated and separated. The amount of hydrate is determined by bromometric titration.

Domange and Longuevalle (*31*) compared several methods for the determination of water in certain products of the French Pharmacopoeia; these methods included drying in an oven, drying in vacuo, and the Karl Fischer procedure.

Eberius (*36*) discussed the various applications of the Karl Fischer reagent to quantitative organic analysis.

Miscellaneous. Gauthier and Maillard (*53*) extended the method of Siggia for the bromometric determination of alkyl sulfides. Hastings (*62*) proposed a spectrophotometric determination of aliphatic sulfides based on the unique ultraviolet absorption bands at 308 mμ exhibited by the iodine complex of aliphatic and acyclic sulfides.

For the determination of menthofuran, Ohloff (*93*) reported a method based on the quantitative reaction with *p*-benzoquinone at 20° C.; after 19 hours, the excess of reagent is determined iodometrically. Naves (*88*) determined menthofuran in peppermint oils by direct infrared absorption measurements at 13.6*M*; the base-line technique should be used. Wegner (*131*) reported that menthofuran gives an intense red color changing to deep violet when treated with the EM reagent. Peppermint oils gave the same color reaction, indicating the presence of menthofuran.

Moster and Prater (*85*) published a rapid spectrophotometric method for the determination of color value of capsicum. The procedure is applicable to the oleoresin. Measurement was made at two wave lengths—569 and 663 mμ or 577.5 and 663 mμ, depending on the color of the extract. A satisfactory spectrophotometric method is much needed; the accepted visual method gives results that are too variable and not easily reproducible.

The Analytical Methods Committee (of England) (*118*) described a method for the determination of lead in foods. After destruction of the organic material, lead was determined with diethylammonium diethyl carbamate. This method appears to be applicable to essential oils.

A series of articles by Cultrera, Buffa, and Trifirò (*16, 17, 27*) dealt with the spectrophotometric analysis of citrus oils. Data concerning ultraviolet absorption were presented for lemon oils as well as mandarin oil and bitter orange oil. The procedure of Sale *et al.* (*105*) for examination of lemon oils by ultraviolet measurement was published; this method was mentioned in the previous review (*59*) without proper bibliographical reference, as at that time it had not appeared in print.

Miller and Kirchner (*82*) gave an excellent detailed report on the use of chromatostrips for identification of the constituents of essential oils, including R_f values for the many systems examined. Labat and Montes (*73*) discussed the use of such chromatostrips and the application to the study of essential oils. Ito, Wakamatsu, and Kawahara (*64*) used chromatostrips for the separation of constituents of Japanese mint oil.

Reitsema (*99*) extended the concept of Kirchner and Miller and developed a "chromatoplate." Using this method he (*100*) examined and classified oils of the Mentha genus. Narodny (*87*) made a preliminary examination of the possible use of chromatography for the evaluation of quality of freshly distilled bay oils of Dominican origin. Using columns of silica gel, empirical data were collected; an attempt was then made to correlate these data with quality as determined by odor.

Zeehuisen (*135*) investigated the application of the vanillin-hydrochloric acid reagent of Rosenthaler to essential oils; he concluded this color reaction was of little value for such complex mixtures as essential oils.

Voge and Sinclair (*130*) discussed odor classifications and suggested their own variation. Such classifications are of little value because they are based on subjective impressions rather than objective physical measurements.

deDortan-Sontag (*32*) presented a brief review of accepted methods of functional analysis as applied to essential oils.

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Clinical Chemistry

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THIS review does not attempt to cover all of the many contributions to quantitative clinical chemistry which have appeared during the past 2 years, up to September 30, 1954. Many of those not mentioned here may prove eventually to be exceptionally significant contributions. However, in the light of the author's present knowledge, and in the opinion of the colleagues with whom he has conferred and to whom he is thus indebted, the contributions mentioned are among the most important.

STANDARD METHODS

The most significant advance for clinical biochemistry during the past 2-year period has been the appearance of the first volume of "Standard Methods of Clinical Chemistry" (*48*). Although this publication presents no new methods or concepts, it is the result of the effort of clinical chemists to arrive at an understanding as to which of several available chemical methods is the best for routine measurement of a given substance or property of clinical importance. Thus an attempt has been made to provide for clinical chemists a compilation of standard procedures equivalent to that which the Association of Official Agricultural Chemists provides in its official publication (*6*) for agricultural chemists. It is obvious that many reviewers feel that the work leaves much to be desired: that the selection of methods presented is not the best (*33*), that purity of reagents to be employed should be specified (*12*), that stability of reagents should be indicated (*8*), that the allowable percentage deviation of duplicates for each method should be specified (*8*), and that qualitative as well as quantitative tests should be included (*35*). It is important, however, that the start has been made.

It is inevitable that the choice of methods is not acceptable to all. In the present volume each selection was made on the basis of the judgment of a relatively small number of clinical chemists. Subsequent volumes will gain in value and recognition as an accepted compilation of standard methods, to the extent to which other clinical chemists effectively voice their constructive criticism regarding the selection of methods and manner of presentation, and the editors and contributors weigh and act on the suggestions of their colleagues, voiced either in personal communications or in the book reviews which follow the appearance of each volume. As the experience of an ever-increasing number of clinical chemists is brought to bear on the subject, the work will acquire greater prestige as a standard compilation until it becomes regarded with an esteem comparable to that in which the AOAC publication is held.

Wherever the contributors and editorial board choose not to follow suggestions made by an appreciable number of readers, it would seem desirable to state in subsequent editions the reasons why, in the judgment of the board, it is deemed unwise to comply. The reading public will then be able to weigh the choices in the light of the reasons given, and to comment further if necessary. The evolution of such a collection of standard methods worthy of almost universal acceptance will be an arduous and continuing but worth-while task. It would appear to this reviewer that it would be helpful to have in such a manual not only the directions for the procedures to be followed, but also numerous notes, in small print, to indicate the probable sources and causes of trouble and means of preventing or overcoming difficulties when these arise. A good example of this may be the type of notes in small print in A. A. Noyes's system of quantitative inorganic analysis (*46*).

Another achievement of the American Association of Clinical Chemists is the launching of its official journal, *Clinical Chemistry*.

A more comprehensive coverage of methods of clinical chemistry has been presented clearly and concisely by Hiller (*27*) for the guidance of technicians. Hepler's book (*26*) gives more attention to the nonchemical procedures of the clinical laboratory.

The last edition of "Practical Physiological Chemistry" (*25*) contains a selection of micromethods for clinical chemists.

VITAMINS AND HORMONES

A new method for the determination of ascorbic acid has proved useful for the measurement of this vitamin in food and pharmaceutical preparations (*53*). It has a high degree of specificity attributable to the reaction of the enediol grouping of ascorbic acid with diazotized 4-methoxy-2-nitroaniline. Dehydroascorbic acid does not interfere. The procedure has not yet been adapted to the measurement of the small amounts ordinarily present in blood, urine, and small samples of tissue, but as the recovery of relatively large added amounts is quantitative (*54*), there is reasonable hope that this reaction may prove useful to the clinical chemist as well as to biochemical investigators. A modification of the more conventional method has been described by Kingsley and Schaffert (*32*).

Recent developments in the field of quantitative determination of steroids have been reviewed extensively (*43, 47*). The methods described, with few exceptions, are not suitable for use in the clinical laboratory. Simpler and more specific methods will need to be developed before it will be practical to assay many steroids in the central laboratory of most hospitals. Some of the methods appear to be well adapted for use in investigational work. It has been widely assumed that the adrenal cortical hormones and their metabolites are either reducing steroids or Porter-Silber chromogens. However, only a small percentage of the administered hormone can be accounted for by steroids which either have appreciable reducing properties or react with Porter-Silber reagent. Fukushima *et al.* (*20*) have found that in neutral ether at least 30% of the metabolites of hydrocortisone were in the form of four new sterols which neither reduce nor react as Porter-Silber chromogens. The failure of the present classical methods to effect sharp fractionation of steroids is indicated by the fact that because of relatively high water solubility some of these new steroids could be detected in the phenolic fraction despite the fact that they are not acidic. The Porter-Silber reaction is reported to yield abnormally high values (*42*) after administration of chloral hydrate, paraldehyde, iodide, or iodine. The chromogens obtained after iodine or iodide yield an absorption curve identical with that derived from hydrocortisone.

The routine determination of chloride has been simplified by Kingsley and Dowdell (*28*) by elimination of the use of thiosulfate. The simpler method for measurement of cholesterol (*30*), mentioned in the previous review (*4*), is proving to be useful and more widely accepted.

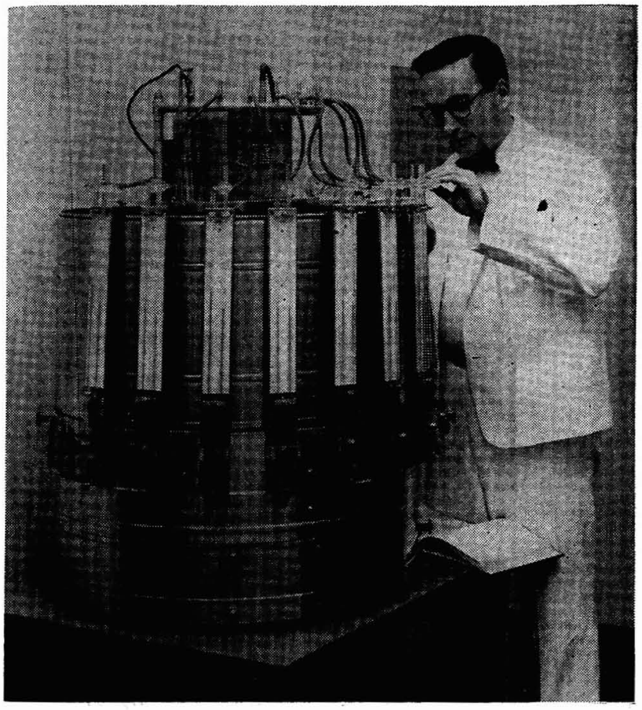
Improved methods for measurement of creatine and creatinine have recently appeared (*48*, page 55; *64*). Sunderman (*61, 62*) and Somogyi (*59*) have improved reagents used for the measurement of blood sugar. The method outlined by Reinhold (*49*, page 65) is a modification of the earlier method of Nelson. Somogyi's studies (*58*) on arteriovenous differences in blood sugar (concentration in fingertip blood—i.e., capillary blood—less concentration in peripheral venous blood) may prove useful in clinical studies. Whether or not Binkley's simple method for the determination of glutathione and its degradation products (*10*) will prove useful in the study of diabetes remains to be seen.

The measurement of adrenogenic amines in shock or for the diagnosis of pheochromocytoma or as aids in the differentiation of conditions involving hypertension has been facilitated by development of methods for ephedrine and norephedrine (*41, 65*). Other methods have been developed for the extraction of noradrenaline and adrenaline and hydroxytyramine from urine (*17*).

Perhaps the best and probably the most widely used method for protein-bound iodine (*7*) is still giving unsatisfactory results in the hands of many operators. Figures for protein-bound iodine, when the procedure is working well, are of considerable clinical significance. However, it is hoped that simpler and more readily reproducible laboratory methods will be developed shortly to yield clinical information of equivalent or greater value.

Flame photometric measurements of sodium, potassium, and calcium have been made more sensitive by the use of photomultiplier tube attachments and a high concentration of acetone in the solutions to be atomized (*29, 31, 66, 68*). The 2- to 20-fold elevation of serum glutamic oxaloacetic transaminase activity observed 12 to 24 hours after myocardial infarction is of interest (*38*). After further investigation measurement of this enzyme might prove to be a useful index of acute tissue damage.

Those concerned with forensic chemistry will be interested in recent methods for the measurement of alcohol (*40, 56*) and mercury (*57*) in blood or urine. The ultraviolet absorption procedures of Goldbaum (*21*) have facilitated differentiation and determination of barbiturates (*1–3*).



Tissue respiration studies form a part of the basic research underlying the development of new drugs and new chemical techniques

Several modifications of the bromosulfaline liver test have appeared. The improvements which they introduce are small compared with the inherent limitations of the procedure, results of which are determined not only by liver function but also by such factors as blood flow in the liver and renal excretion of dye.

Apparently improved methods have appeared for the measurement of serum cholinesterase (*13, 49*), plasma heparin (*19*), cystine and cysteine (*14*), choline (*44*, page 265), and nucleic acids (*44*, page 287). Considerable progress is being made in the fractionation of protein mixtures (*67*) and in the specific determination of the various proteins so separated (*50–52*). It seems reasonable to expect that the detection and identification of abnormal hemoglobins may become a matter of considerable clinical significance, now that the work of Itano and his colleagues (*24*) has facilitated such detection.

NEW TECHNIQUES

The location of the boundary between the realm of relatively simple, straightforward methods which are generally regarded as capable of yielding results of clinical importance, and on the other hand, the methods which are useful primarily in investigative work, will vary somewhat from one laboratory to another, depending upon the competence and interest of the technical staff and the interests and demands of the clinical investigators. Methods which are generally regarded as too complicated or exacting for a general clinical laboratory, or methods which yield results of as yet unproved clinical significance, will probably be conducted by the general clinical laboratory staff in locations where the clinicians are interested in pursuing a related investigation. Although many of these will be dropped when lack of clinical significance of results is recognized, nevertheless from this group will come new procedures which will yield information of very definite clinical significance, and will replace some of the standard methods now generally employed.

It appears most likely that these new and promising techniques will use some of the very potent tools of fractionation which have been exploited to excellent advantage during the past several years (*34*). Countercurrent fractionation has made possible improved methods of measuring estrogens in biological material (*16, 45*). Starch block (*44*, page 141) and paper (*15, 22*) zone electrophoresis (*18*) have permitted the separation and identification of complex lipoproteins (*37*), enzymes (*5*), and other protein components of mixtures (*15, 36, 39*), and separation of amino acids (*15, 22*) and iodine-containing derivatives, precursors, or metabolites of thyroxin (*15*). Paper chromatography continues to be exploited to good advantage for fractionation of barbiturates (*1, 2*), hemoglobins (*24*), urine sugars (*44*, page 205; *60*), thyroid (*23, 44*, page 243), and adrenal hormones (*11, 44*, page 171). These methods have been improved by the use of more or less specific stains for given types of compounds—e.g., Sudan black for lipides (*37, 63*), periodate followed by Schiff's reagent for carbohydrates (*36*), and bromophenol blue for proteins.

Tests which employ radioactive isotopic labels were once considered as scarcely falling within the scope of the work of the clinical laboratory. However, in the not too distant future, use of these is likely to become sufficiently widespread and helpful to justify training the technical help of the clinical laboratory to handle at least the biological material from patients subjected to tracer studies, if not the undiluted solutions of radioactive isotopes. The clinicians responsible for the ordering and administration of the tests should be well versed in the limitations of the tests, the possible fallacies of the interpretation, and the need to avoid exposure of the patient and personnel to hazardous doses of radiation. Good preparations of iodine-131–labeled albumin and gamma-globulin are now available and are admirably adapted to the determination of plasma volume (*9, 55*).

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Water Analysis

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THIS sixth review of analytical procedures applied to the analysis of water reported in the technical literature covers a 2-year period. Five previous annual reviews were published in ANALYTICAL CHEMISTRY in 1949 (*131*), 1950 (*132*), 1951 (*133*), 1952 (*134*), and 1953 (*135*).

Of considerable significance in this review is the large number of papers dealing with the radioactivity of water. There is considerable interest in isotopic analysis for hydrogen-2, hydrogen-3, and oxygen-18. Papers on instrumental analysis show continued activity in the development of instrumentation, particularly in the field of polarography. Polarographic methods are becoming more common in the determination of dissolved oxygen, residual chlorine, nitrite, nitrate, and iodide.

LITERATURE REVIEWS AND SCHEMES OF ANALYSIS

A panel chairmaned by Heukelekian has prepared literature reviews of industrial waste waters in 1952 (*86*) and 1953 (*87*). The section on analytical methods covers determination of biochemical oxygen demand, oxygen consumed, dissolved oxygen, organic compounds, nitrogen compounds, metallic ions, halogens, sulfur compounds, phosphorus compounds, cyanogen compounds, and instrumentation. Over 100 references are cited in the two reviews.

A review of water chemistry and water treatment was published by Zehender (*247*).

A systematic scheme for analysis of industrial water and industrial waste water has been presented by Phillips and Stoffer (*173*). An outline is given for a scheme embracing over 40 constituents and properties of industrial water. The two main divisions of the scheme are listed. One deals with constituents affected by contact with air and the other deals with those not so affected.

INSTRUMENTATION

Several significant papers were presented at the Symposium on Continuous Analysis of Industrial Water and Industrial Waste Water. One of these, by Rosenthal (*194*), covered some of the electrical measurements in water analysis such as the determination of pH, electrical conductivity, and redox potentials. Recent developments in these fields were reviewed. It was pointed out that determinations based on oxidation-reduction potentials were useful in the determination of residual bromine and chlorine and such ions as chromate, but that the measurements involved are not simple and correlation of the data is difficult. A paper given at the symposium by Madarasz (*140*) reviewed the available techniques for automatic sampling procedures and considered the requirements for obtaining a truly representative sample for analysis.

Diskant (*46*) has presented a paper on photometric methods of water analysis, in which the sample is taken through all the phases involved in the analysis, starting with sample collection and carrying through to the final development of the color. The necessity for "fixing" the sample immediately after collection is pointed out. Wyatt (*246*) has reviewed instrumentation for water laboratories with consideration given to the concentration regions to which specific instrumental methods are applicable.

Most of the instances of the use of high frequency titration have been at concentration levels considerably higher than are encountered in water analysis; however, Bien (*20*) recently applied the method in the form of a microtitration to studies of sea water. The analysis involved the determination of chloride and sulfate in interstitial water of marine sediments and only 0.3 to 0.4 ml. of sample was available for analysis. Dioxane was added to the samples to lower the dielectric constant and thus improve the sharpness of the end point. Reilly and McCurdy (*186*) have reviewed the principles of high frequency titrimetry.

Other papers of interest to people working with instrumental analysis include that of McBryde (*137*), which considers errors in photometric determinations, and a paper by Creamer and Chambers (*38*), which describes the construction of an inexpensive conductivity bridge built around a vacuum tube voltmeter. Knowlton (*113*) has described the continuous monitoring of fluoride compounds added to water by means of an automatic recording conductivity meter. Murray (*156*) has considered the application of physicochemical methods for the determination of several constituents. Nitrite is determined by adding the sample to a potassium iodide solution followed by measurement of the potential of the iodine-iodide electrode. Dissolved oxygen is determined similarly. Sulfide is determined by means of the catalytic effect on the azide-iodine reaction. Nitrogen is evolved, which is measured manometrically.

ALKALI METALS

Flame photometry is the dominant method of determining sodium and potassium. Reviews on the subject have been presented by Smit (*213*) and Schuhknecht (*200*). Mehlich and Monroe (*149*) studied the flame photometric determination of potassium in soil extracts with a view to identifying the cause of certain discrepancies in results between laboratories. The concentration range studied includes that normally encountered in waters for potassium and several analytical points of interest to water chemists are brought out.

The triple acetate determination of sodium was studied by Rogers and Haight (*191*), who were interested in the effect of the addition of alcohol to suppress solubility. It was found that the precipitate contained appreciable amounts of alcohol, dependent on the nature of the divalent ion in the triple acetate salt. Shigematsu and Kimura (*206*) studied the use of the nickel uranyl acetate reagent in the determination of sodium. Navone (*164*) has described an ion exchange method for the determination of sodium, for application to waters which are not complex in nature. Three determinations must be made: total cation by ion exchange, alkalinity, and total hardness. Sodium is then found by a difference calculation.

ALKALINE EARTH METALS

The pervading influence of radioactivity has appeared in connection with the determination of strontium concentrations and ratios of strontium to calcium in natural waters. A spectrographic study of the strontium and calcium concentrations in water samples from many areas of the United States was carried out by Alexander, Nusbaum, and MacDonald (*2*). A modification of the copper spark method was applied using molybdenum as the internal standard. A sodium chloride buffer was added, which approximately equalized salt concentration. Spectrographic determinations of calcium and magnesium were compared against the flame photometer and Schwarzenbach titration methods with generally satisfactory agreement. The results of the strontium determinations on the waters of many city supplies are given. Meloche and Shapiro (*150*) applied the spectrograph to the analysis of calcium and magnesium in lake waters using a rotating silver electode. Concentrations covering the range 0.1 to 30 p.p.m. were analyzed with manganese as the internal standard.

As has been the rule in recent years, a great number of papers have appeared on the application of complexometric titrations with the reagents brought into use by Schwarzenbach and his coworkers. Many of the papers on this subject are repetitious or present minor modifications of doubtful significance. Only the more significant papers of the large number available are considered here. Schwarzenbach (*202*) has reviewed the principles of the complexometric titrations in a paper entitled "Chelate Complex Formation as a Basis for Titration Processes." The theory of complexometric titrations is elucidated from the standpoint of considering the metal ions as Lewis acids. The reaction of a metal ion with a polydentate group such as is found in ethylenediaminetetraacetic salts is shown to be analogous to the neutralization of an acid with a base. The relative stability of the metals complexed with polydentate molecules is considered and the important analytical implications of the stability differences are pointed out. Most of the applications of complexometric titrations to water analysis have involved metal indicators for detection of the end point. Schwarzenbach points out that other phenomena such as pH effects can be used as well.

The use of ultraviolet spectrotitration for the determination of calcium, magnesium, and other metals with ethylenediaminetetraacetic acid was reported by Sweetser and Bricker (*221*). The metal complexes show little absorption above 222 mμ, whereas reagent shows strong absorption up to about 245 mμ. On titrating at 228 mμ a sudden increase in absorption is observed at the end point. Laitinen and Sympson (*122*) have applied ethylenediaminetetraacetic acid to a polarographic titration of calcium in which the reaction of excess ethylenediaminetetraacetic acid with zincate is used to indicate the end point. Přibil (*181*) has studied the masking of interfering metals in the complexometric titrations and found triethanolamine and cyanide to be effective. Another study of interferences in the ethylenediaminetetraacetic acid titration was presented by Cheng, Melsted, and Bray (*32*), who used dimethyldithiocarbamate followed by isoamyl alcohol extraction to separate manganese, iron, and copper. Although the work was done in connection with soil analysis, the procedure should be applicable to the removal of the same interfering ions in water. Brunisholz, Genton, and Plattner (*26*) used ion exchange to remove phosphate prior to titration with ethylenediaminetetraacetic acid. Magnesium interference in the calcium titration was eliminated by adding excess ethylenediaminetetraacetic acid and back-titrating. Brooke and Holbrook (*25*) also applied ion exchange for the removal of polyphosphate interference. The complexometric determination was applied to brines and highly saline deposits by de Sousa (*44*).

Several papers are reported on the colorimetric determination of calcium and magnesium. Williams and Moser (*242*) used ammonium purpurate for the colorimetric determination of calcium. Harvey, Komarmy, and Wyatt (*79*) developed a colorimetric determination of magnesium with Eriochrome Black T. Calcium is first removed by precipitation as the sulfate in 90% alcohol. Dirscherl and Breuer (*45*) determined magnesium similarly, using oxalate to precipitate calcium. Glemser and Dautzenberg (*61*) studied the colorimetric determination of magnesium with Titanium Yellow.

ALUMINUM AND IRON

Simons, Monaghan, and Taggart (*210*) used *o*-phenathroline and Pontachrome Blue Black R for the determination of iron and aluminum in ocean waters. It was reported that no interferences were encountered in the application of these determinations to sea waters. Ashizawa (*6*) determined iron and aluminum in natural waters using an amyl alcohol extraction to separate the iron as the thiocyanate and salicylic acid to separate the aluminum. Aluminum was then determined with aluminon. It was stated that the interference of fluoride in the colorimetric determinations could be removed by the addition of thorium nitrate. Quentin (*183*) used paper chromatography for the separation of aluminum in mineral waters. The aluminum was then eluted with hydrochloric acid and determined colorimetrically with aluminon. A polarographic determination of aluminum in sea water was reported by Ishibashi and Fujinaga (*104*). A mixed iron-aluminum precipitate was obtained in the initial step and the iron was then removed in a Melaven cell to prepare the solution for polarographic determination of the aluminum.

Smith and McCurdy (*214*) reported the application of 4,7-diphenyl-1,10-phenanthroline to the determination of iron in raw and treated municipal water supplies; the sensitivity is approximately twice that of *o*-phenanthroline and interferences are not significant. Lieber (*127*) designed a field test for iron based on the thiocyanate method. Wilkins and Smith (*241*) described the simultaneous determination of iron and copper using *o*-phenanthroline.

COPPER, ZINC, AND LEAD

The familiar diethyldithiocarbamate method was used by Chow and Thompson (*36*) for the determination of copper in sea water; the reagent was found superior to dithizone. Samples of 500 ml. were collected in polyethylene bottles and the carbamates were extracted with xylene. No significant interferences were encountered. Jenkins (*107*) used the carbamate method to determine traces of copper at the 0.001 p.p.m. level in highly purified water. Noll and Betz (*168*) reduced interference in the carbamate procedure by using a lower indicator concentration.

The cuproine determination of copper was studied by Hoste, Eeckhout, and Gillis (*95*), who give analytical procedures for several materials including natural waters. Smith and Wilkins (*215*) presented a study of the properties of bathocuproine, a new copper reagent said to be superior in several respects to cuproine. The determination of copper with neocuproine was investigated by Gahler (*57*), who found that the color complex could be completely extracted from 125 ml. of aqueous phase by as little as 10 ml. of chloroform with only 30 seconds' agitation. Cyanide and sulfide interfere markedly. Feigl and Caldas (*48*) have introduced a new copper reagent, alizarin blue, said to be more sensitive than any yet reported. It is stated that copper concentrations as low as 0.001 p.p.m. can be readily detected.

The determination of zinc with dithizone was modified by Vallee (*230*) to eliminate the extraction step. Methyl Cellosolve is added to the sample to make a monophase water-organic system. Kato and Takei (*109*) used cuprethol to complex cadmium and bismuth and thus eliminate their interference in the dithizone zinc determination. Hermanowicz and Sikorowska (*85*) applied a paper chromatographic technique to the determination of zinc concentrations as low as 0.05 p.p.m. in natural water. The color was developed with dithizone. Mayne and Noordhof (*145*) used sodium diethyldithiocarbamate to determine zinc in sea water. The use of polyvinyl alcohol as the protective colloid was said to be much superior to gum arabic.

Berger and Pirotte (*19*) studied the dithizone determination of lead, and presented two spectrophotometric procedures. The stability of trace concentrations of lead in water under varied pH conditions was studied by Sushinskaya (*219*). It is reported that lead is not absorbed in acid or neutral solution but is rapidly taken up by clay particles at pH values greater than 8.

TRACE METALS

A considerable amount of study has been given to the use of the spectrograph for the determination of trace elements in various types of natural water. Heggen and Strock (*82*) have reported on a spectrographic procedure that has been used for several years in the laboratories of the Saratoga Springs Research Commission of New York State for the determination of trace metals in brines and fresh waters. For the initial separation and concentration of the desired metals a mixed reagent of 8-quinolinol, tannic acid, and thionalide is used. Fourteen metals are determined with indium as the internal standard. Wilska has given a great deal of study to the spectrographic determination of trace metals in water, using the porous cup electrode technique (*243*, *244*). Collection and concentration techniques are sometimes used, but in general direct spectral analysis of the solution is satisfactory. Description of sample collection and preparation of containers and other essential details are given. The results of analyses of a wide variety of surface and ground waters are reported. Pohl (*176*) has reviewed the methods applicable to the spectrographic determination of trace metals in water and reported on the various concentration techniques available. A review of the spectrographic techniques applicable to sea water was published by Hitchcock and Starr (*90*). The rotating disk, flame excitation, porous cup, impregnated electrode, open-cup residue, and briquet excitation methods were considered; in general the methods are applicable to the determination of calcium, sodium, magnesium, potassium, strontium, and aluminum by direct determination but prior concentration is usually required for iron, copper, barium, lithium, and silver.

Chromatographic techniques were applied to natural water by Carritt (*31*), who used a dithizone column on a cellulose acetate base for the separation of lead, zinc, cadmium, cobalt, copper, and manganese. Concentration factors of up to 1000 were achieved in some cases. The metals were diluted with hydrochloric acid and ammonia. Ashizawa (*4*) used chromatographic analysis for the determination of copper, zinc, nickel, and cobalt in natural water at concentrations in the range of 1 γ per liter. Determination of uranium, vanadium, and chromium were made by Harasawa and Sakamoto (*77*) by chromatographing in an acetone–acetic acid–water system.

The determination of minute traces of arsenic in sea water is a difficult chemical problem. The determination was made by Smales and Pate (*211*) by the use of radioactivation. The sample was activated by slow neutron irradiation and the arsenic activity was separated chemically with a carrier and was measured and characterized by the usual radiometric methods. Concentrations as low as 2.6 γ per liter were determined. Ashizawa (*5*) determined antimony in hot spring waters, using hydrogen sulfide as the colorimetric reagent and polyvinyl alcohol as the supporting medium.

Misumi (*154*) reported the determination of silver in hot springs using a coprecipitation with tellurium as the gathering step. The precipitate was dissolved in nitric acid and the silver was determined colorimetrically according to the method of Sandell and Neumayer. Titanium in mineral waters was determined by Kimura, Ikeda, and Watanabe (*111*) with hydrogen peroxide following a concentration step by iron precipitation. Hluchán and Mayer (*91*) described the determination of manganese with ammonium persulfate. Ishibashi and Shigematsu (*105*) determined chromium in sea water by means of the color reaction with diphenylcarbazide. The chromium was gathered by coprecipitation with aluminum hydroxide and the precipitate was taken up in a flux. Vanadium interference was removed by extraction of the metal.

The general subject of the determination of submicrogram amounts of metals in macro volumes of solution was reviewed by Rogers (*190*), who considered the concentration techniques such as carrier precipitation, extraction, chromatography, and ion exchange, and the modern instrumental analytical techniques. Haberlandt and Scheminzky (*74*) reviewed the trace analytical methods applicable to mineral water. Gillis (*60*) has discussed the sensitivity of analytical reactions and has cited several specific and sensitive analytical reagents suitable for direct tests.

CARBON DIOXIDE AND SILICA

A method for the determination of low concentrations of carbon dioxide in water was given by Smith, Gilbert, and Howie (*216*), who improved the sensitivity of Clarke's procedure by using a larger sample and devising a more rigid protection against atmospheric contamination. The determination is sensitive to concentrations in the region of 0.1 p.p.m. The efficiency of the carbon dioxide free blank determination was tested with a carbon-14 tracer technique. Saruhashi (*198*) determined the total carbonate concentration of sea water by a microdiffusion technique in a 6-cm. concentric compartment diffusion cell.

Chow and Robinson (*34*) conducted research on the forms of silica determined by the colorimetric molybdate procedure in natural waters. It was found that low pH, high silica concentration, and low salt concentration reduce the apparent silica recovery. The results are attributed to a silicic acid–silicate ion equilibrium state. Piryutko and Shmidt (*175*) on the other hand attributed low silica recovery to a monomer-polymer equilibrium for silica in natural water. Converting the polymer to a monomer by chemical treatment with sodium hydroxide or hydrofluoric acid increases the per cent recovery. Katsura and Tarutani (*110*) studied the particle size of colloidal silica in relation to the reactivity with molybdate, and found that particles larger than 2 or 3 microns did not give an ammonium molybdate reaction.

CHLORIDE, BROMIDE, AND IODIDE

Most of the reported studies of the chloride determination centered around the mercuric nitrate titration. Gad and Manthey (*56*) studied the method in detail with particular attention to interferences. They have presented a table representing the approximate upper limits of tolerable metal concentrations (parts per million).

Zn	100	Cr	100
Pb	100	Cu	50
Al	100	CrO_4	10
Ni	100	Fe	5

Vetrov (*233*) carried out a critical study of the mercurimetric determination and pointed out several precautions which must be observed. Stability of the mercuric nitrate is improved by adding nitric acid to make the solution 0.01 to 0.2N and by presence of metallic mercury. The titration should not be made in direct sunlight. Iron and sulfate interfere. The indicator should be added toward the end of the titration. A preliminary Mohr titration is often necessary. Thomas (*225*) also reported on the mercurimetric titration and Smit (*212*) used the method for determining chloride in condensates. Ikeda and Komooka (*101*) made a study of the Mohr titration and reported interference on application to hot springs due to the high sulfate content. Sulfide also interferes. The addition of an equal volume of ethyl alcohol eliminates sulfate interference and cadmium carbonate eliminates the effect of sulfide.

The amperometric titration of iodide was investigated by Kolthoff and Jordan (*115*) using the rotating platinum electrode. Several systems were developed, one of which was sufficiently sensitive for water analysis. This involves the oxidation of iodide to iodate with chlorine water and subsequent titration with potassium iodide. A different form of amperometric titration was used by Kramer, Moore, and Ballinger (*116*), who applied the Wallace and Tiernan amperometer to the determination of iodides in the range 0.2 to 2 p.p.m. Phenyl arsene oxide was used as the titrant. Davidyuk (*41*) used a potentiometric titration with bromate for the determination of small quantities of iodide in natural water. A spectrophotometric determination was used by Pirotte and Berger (*174*), who applied the catalytic effect of iodide on the ceric oxidation of arsenite to the determination of trace quantities. The absorption of ceric ion is read at 375 mμ at 5 and 15 minutes.

The determination of iodide and bromide in natural waters by successive thiosulfate titrations was applied by Höfer (*93*). Aleksandrov and Levchenko (*1*) presented a somewhat different system of consecutive titration for iodide and bromide, in which the iodine is liberated and titrated with thiosulfate and the bromide is then titrated after the addition of sulfuric acid and ammonium molybdate. Ballczo and Mondl (*12*) described methods of column enrichment and separation for chloride, bromide, and iodide. Iodide is collected on a silver bromide column, bromide on a silver chloride column, and chloride on a silver chromate column.

FLUORIDE

As the general adoption of fluoridation for municipal supplies progresses, the problems of control of fluoridation become increasingly more pressing. Jacobson (*106*) has reviewed the current analytical situation with respect to fluoride and has concluded that none of the colorimetric, spectrophotometric, or volumetric procedures available to date are entirely satisfactory for control purposes. A spectrophotometric method recently reported by Megregian (*147*) looks promising as a possible solution to the problem. The method, which is based on the bleaching of zirconium–Eriochrome Cyanine R lake, claims immediate color development, simplicity, and relative freedom from interference. The phosphate interference is low. Sulfate interference is significant but easily corrected. The sensitivity is more than adequate. Beer's law is followed at concentrations up to 1.2 p.p.m. of fluoride.

Many other colorimetric methods have appeared in the literature. Nichols and Condo (*165*) studied the organoferric complexes as fluoride indicators. They concluded that 5-phenylsalicylic acid and resacetophenone were superior and determinations based on these reagents were presented. Interferences are considerable. The aluminum-hematoxylin test for fluoride was studied by MacNulty and Hunter (*139*). Revinson and Harley (*187*) devised a colorimetric procedure based on the thorium–chrome azurol S reaction. The determination is reasonably sensitive, but interferences are serious. Babko and Khodulina (*10*) used the titanium–chromotropic acid system in a chloracetic acid buffer. Phosphate interferes. Venkateswarlu and Ramanathan (*231*) used the thorium nitrate titration for the estimation of fluoride in potable water. Bond and Murray (*21*) studied the thorium nitrate titration extensively. Alizarin was used as the indicator. Calcium, phosphate, and sulfate interfere.

Lambert (*123*) used a rather different approach to the problem by using the accelerating effect of fluoride on the ceric-iodide reaction as a measure of the fluoride concentration. The time required for the appearance of a starch-blue color is proportional to the fluoride concentration. Another paper by Lambert (*124*) described a determination based on the thorium-amaranth lake. Powell and Saylor (*180*) developed a fluorometric determination using the aluminum complexes of Eriochrome Red B and Superchrome Garnet Y. Although the reagents are sufficiently sensitive for water analysis, the susceptibility to interferences is considerable. The familiar zirconium-alizarin method was studied by Shaw (*205*) and Tedesco (*223*). Grutsch and coworkers (*71*) attempted to eliminate some of the interferences in the zirconium-alizarin method by the addition of thioglycolic acid. It is claimed that this procedure eliminated the interference of iron, manganese, and residual chlorine. Megregian and Solet (*148*) and Frazier and Oldfield (*51*) studied the improvement of fluoride distillation techniques.

COMPOUNDS OF SULFUR

A new reagent for the volumetric determination of sulfate was introduced by Belcher, Kapel, and Nutten (*15*), who used 4,4-diaminotolan in place of benzidine for the precipitation of sulfate prior to the titration. Toennies and Bakay (*226*) studied the turbidimetric determination of sulfate critically and recommended an ethyl alcohol–dipropylene glycol mixture for use as the suspending agent. They devised a procedure which is stated to be accurate over the range 0.1 to 100 p.p.m. of sulfate. Another turbidimetric procedure was presented by Iljin (*102*), who used a hydrochloric acid–sodium chloride medium for the development of the turbidity. Additional data on nephelometric sulfate determinations were given by Günther (*72*). Spillner and Voigt (*217*) described a conductometric sulfate determination using barium nitrate. The author used an "overtitration" procedure which gave results in 3 minutes under very carefully standardized conditions.

The polarograph was applied to the determination of sulfate in mineral waters by Matsumoto and Shibayama (*144*). By means of lead nitrate reagent sulfate, sulfide and total sulfur could be determined. Geilmann and Bretschneider (*58*) used the chromate method for the determination of low concentrations of sulfate. Hunt (*97*) utilized ion exchange to remove cations and thus improve the volumetric sulfate determination with ethylenediaminetetraacetic acid.

Mikula (*153*) devised a determination of sulfite said to be more specific than the iodometric determination. It is based on the use of fuchsine, which is reduced to a colorless compound by sulfite. Mueller and Rand (*155*) developed a qualitative field test for sulfide based on Raschig's azide-iodine reaction. Bubbles of nitrogen form in the sample contained in a watch glass if sulfide is present in concentrations exceeding about 0.1 p.p.m.

COMPOUNDS OF NITROGEN

The polarographic method of nitrate determination was studied by Rand and Heukelekian (*184*) and applied to well waters and sewage. The determination is based on measurement of the difference in the diffusion current at a potential of 1.2 volts before and after the addition of ferrous sulfate. The supporting electrolyte is zirconyl chloride. Lawrance and Briggs (*125*) have applied an essentially similar polarographic nitrate method and have pointed out some of the precautions that must be observed in applying the method to natural water. Slime and algae completely suppress the nitrate wave and must be removed. The authors used aluminum flocculation for this. Trace concentrations of hydrogen sulfide invalidate the analysis. Iron and several other substances interfere. The authors stress the importance of "fixing" the nitrate sample immediately after collection to halt the progress of bacterial action. Chloroform was used successfully.

The colorimetric methods for nitrate determination have received some attention. Sijderius (*207*) modified the phenoldisulfonic acid determination by adding the reagent before evaporating the sample to dryness. It is claimed that this procedure eliminates chloride interference up to fairly high concentrations. Hahn (*75*) used ethylenediaminetetraacetic acid to eliminate the precipitation of calcium and magnesium in the phenoldisulfonic acid determination of nitrate. The complexone is introduced by way of the base used for neutralization. Armondola (*3*) has studied the brucine method of Noll and made certain modifications. Machida (*138*) applied the reduced strychnine method to the determination of nitrate in sea water. He prepared the reagent by a new method involving the use of liquid zinc amalgam. A procedure for use of the reagent in a photometric analysis is given.

The polarograph has also been applied to the determination of nitrite. Chow and Robinson (*35*) determined nitrite polarographically in the presence of molybdate. Two electrolyte systems are described for high and low concentrations of nitrite, with about 1 p.p.m. as the concentration limit for the more sensitive of the two systems.

The familiar Griess reagent for the colorimetric determination of nitrite has been modified considerably by Benschneider and Robinson (*17*), who substituted sulfanilamide and *N*-(1-naphthyl) ethylenediamine for the usual reagents. The color develops rapidly and is reported to be stable for 2 hours. Beer's law was followed in applications to sea water analysis. Pappenhagen and Mellon (*172*) eliminated the coupling step of the Griess reaction by utilizing the ultraviolet spectral region for measurement of the absorbancy of the nitrite reaction product formed with sulfanilic acid. Braarud and Føyn (*22*) reported a nitrite concentration survey of polluted sea waters in Oslo Fjord. The standard Griess procedure was used. It was found that oxygen-rich water rapidly oxidizes ammonia to nitrite in the presence of active bacteria. Ikeda (*100*) carried out a determination of nitrite in mineral waters containing high concentrations of hydrogen sulfide. Cadmium carbonate was used to eliminate sulfide interference. Woodward (*245*) used the Griess determination for both nitrite and nitrate. Nitrate was reduced prior to colorimetric determination by the aid of metallic zinc in a solution of pH 4 to 5.

Ammonia in natural waters was determined by Riley (*188*) using the sodium phenate–hypochlorite colorimetric reaction of Russell. The determination can be applied directly to most fresh waters, but prior distillation is required for sea waters. Hydroxylamine and copper interfere, but hydrazine does not. Sijderius (*208*) devised a photometric version of the Nessler test for ammonia and Batmanova (*13*) prepared a stable solid mixture of the reagent for use in field work. Tanaka (*222*) studied the occurrence of hydroxylamine in lake water as an intermediate in the bacterial reduction of nitrate. An increase of the hydroxylamine and nitrite concentration with depth was noted and was correlated with a regular decrease in nitrate concentration with depth.

RESIDUAL CHLORINE

The increasing popularity of the instrumental methods for the determination of residual chlorine is very much in evidence. Clark (*37*) has described the determination of free and combined chlorine with phenylarsene oxide by amperometric titration. Monochloramine is determined at pH 7 by the addition of potassium iodide and dichloramine by the same method at pH 4. Griffin (*68*) in a paper presented at the Symposium on Continuous Analysis of Industrial Waste Water reviewed the shortcomings of the colorimetric determinations of residual chlorine and considered the advantages of the instrumental methods for continuous monitoring of chlorine. For the determination of combined chlorine potassium, iodide must be added to the flow ahead of the cell. Marks and Chamberlain (*141*) reported on the application of amperometric methods to the determination of chlorine in plating wastes where the metal concentration is sufficient to upset some of the colorimetric methods. It was stated that chlorine concentrations as low as 0.2 p.p.m. could be determined amperometrically. Day, Horchler, and Marks (*42*) compared the *o*-tolidine method with two amperometric methods on three different types of sewage and reported that the instrumental method generally gave higher residual chlorine results.

Palin (*171*) proposed a volumetric determination of chlorine based on the use of *o*-tolidine and ferrous ammonium sulfate at a controlled pH. Mono-, di-, and trichloramines may be determined by a difference titration procedure. Belcher, Nutten, and Stephen (*16*) have recommended the use of 3,3′-dimethylnaphthidine as a colorimetric reagent, and state that the reagent is superior to *o*-tolidine in sensitivity, color stability, and ease of color measurement. A purple-red color is produced which will detect as little as 0.05 p.p.m. of chlorine. Asmus and Garshagen (*8*) used barbituric acid for the residual chlorine determination by photometric means. Cyanogen chloride is first formed, which reacts with a mixture of pyridine and barbituric acid. A similar procedure using pyridine and a primary or secondary amine was described in an earlier paper (*7*). Lipps and Gaertner (*128*) reported some modifications in the *o*-tolidine and the dimethyl-*p*-phenylenediamine colorimetric determinations. Gad and Manthey (*55*) reported the use of a proprietary reagent mixture of sodium arsenite and methyl orange for the determination of chlorine. Goris and Hauss (*65*) modified the *o*-tolidine determination for photometric purposes. Hodgden and Ingols (*92*) reported the use of tyrosine in a determination of chlorine dioxide. It is stated that chloramines, hypochlorous acid, and manganese do not interfere.

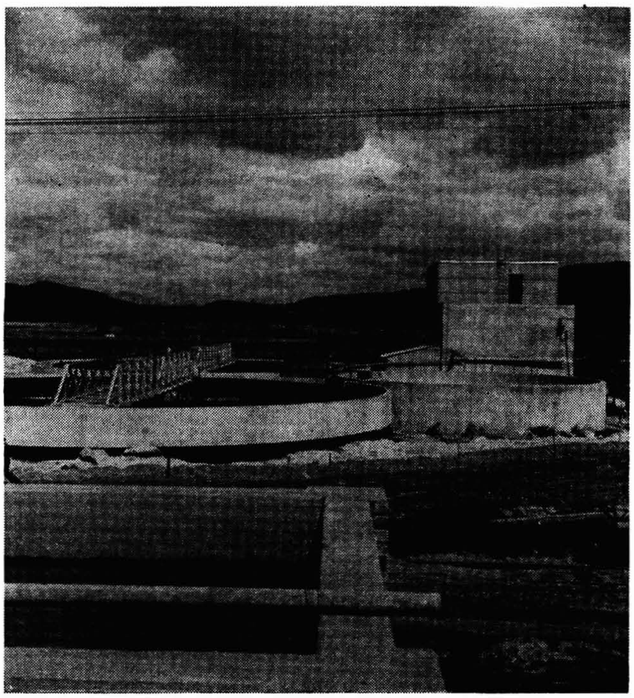
PHENOL

An absorptiometric determination of phenol using the ultraviolet region was presented by Smauch and Grubb (*199*). The principle, previously reported by Murray (*157*), is based on the shift in ultraviolet absorption of phenols under the influence of pH change. The determination is made in tributyl phosphate medium, as this solvent is transparent to ultraviolet. Interfering oils are removed by extraction, phenol is separated by tributyl phosphate extraction, the extract is divided into two portions of pH 5 and 12, and the absorbance difference between the two is measured at 301 mμ. Hill and Herndon (*88*) used *p*-nitrosodimethylaniline for the phenol determination. A blue color develops which is sensitive to 5 p.p.b. of phenol. Nusbaum (*169*) modified the method of Hill and Herndon by use of the oxalate salt for preparation of the reagent to improve the stability. Potassium ferricyanide catalyzed the reaction and eliminated sulfide interference. Deinum and Hanssen (*43*) used a colorimetric phenol determination involving the addition of *m*-nitroaniline, sodium nitrite, and reagents to adjust the pH. The color is said to be stable for 24 hours. Gorbach and Rohner (*63*) used diazotized nitroaniline followed by addition of bromate-bromide solution and potassium iodide with titration of liberated iodine. Noisette (*167*) studied the diazo procedure and the Gibbs procedure and drew comparative conclusions. The Gibbs method is the more sensitive but is subject to many interferences. The diazo method is sensitive to 0.02 p.p.m. It is stated that phenol determinations should be made within 6 hours after sampling, unless the sample has been fixed with copper.

The report of the Subcommittee on Refinery Effluent-Water, Analytical Methods Committee, of the American Petroleum Institute (*80*) has reviewed the progress made in the phenol determination as a result of the subcommittee's activities in the field of effluent water analysis. The infrared method of Simard and coworkers (*209*) and the ultraviolet method of Smauch and Grubb referred to above are the direct result of the work of this subcommittee.

ORGANIC COMPOUNDS

The problem of the industrial pollution of streams with organic waste to produce toxicity and objectionable tastes and odors is continually becoming more serious. The Subcommittee on Refinery Effluent-Water, Analytical Methods Committee, American Petroleum Institute, has made considerable strides in attacking the petroleum industry phase of the problem. In a recent report (*80*), the subject of qualitative and quantitative analysis for the organic constituents of refinery wastes was reviewed. Four methods were cited as applicable to waste concentrations in the 100 p.p.b. range, infrared absorption analysis as described by Simard and coworkers (*209*), benzene extraction by the method of Musante (*158*), reflux distillation followed by benzene extraction, and the extraction-pycnometer method. A fifth method which is said to be applicable in the 10 p.p.b. range is afforded by the use of the mass spectrometer. Melpolder, Warfield, and Headington (*151*) have carried out some pioneering work in the application of this instrument to water analysis, and have developed a procedure which involves stripping the hydrocarbon fraction from the sample with a stream of hydrogen, condensing the hydrocarbons in a liquid nitrogen trap, and analyzing the separated hydrocarbons in the mass spectrometer. Both qualitative and quantitative results are obtained.



COURTESY H. K. FERGUSON CO.

Large amounts of cold water, taken from nearby streams, are purified in this plant for use in manufacture of caustic soda

The Sanitary Engineering Center Group has continued its work on systematic organic analysis, and two reports of progress are available. Middleton, Braus, and Ruchhoft (*152*) reviewed the activated carbon filter technique for sample collection in streams, and described the use of infrared for analysis of the extract from the columns. Braus, Middleton, and Ruchhoft (*24*) reported on developments in their scheme of analysis of organic matter by solubility differences. The compounds are broken down into four categories: phenolic compounds, acidic compounds, amphoteric compounds, and water-soluble nonvolatiles. Infrared analysis is applied to the individual groups. The determination of pyridine and pyridine base compounds was studied by Kroner, Ettinger, and Moore (*117*), who used the Koenig reaction for the development of a colorimetric method. The procedure involves distillation of the sample followed by reaction with benzidine and cyanogen bromide in the presence of a layer of *n*-butyl alcohol. The method is reported to be sensitive to pyridine concentrations as low as 0.005 p.p.m.

Wedgwood and Cooper (*235*) used infrared analysis for the determination of polynuclear hydrocarbons in industrial effluents. A cyclohexane extract was chromatographed on an alumina column and 100 fractions were collected for optical analysis. Tables of optimum wave lengths for 16 common polynuclear hydrocarbons are given. Medin and Herndon (*146*) published a colorimetric procedure for the determination of naphthalene in industrial wastes. The procedure is similar to that of Schwartz, in which anhydrous aluminum chloride in chloroform is used. A blue color develops, which is measured at 525 mμ. Sutton (*220*) developed a colorimetric determination of pine oil using vanillin–hydrochloric acid reagent. The reagent actually determines α-terpineol, the main constituent of pine oil. Therefore an empirical concentration curve must be prepared for each type of pine oil encountered. Berck (*18*) used the Schechter-Haller method for the determination of DDT concentrations as low as 1 γ per liter. It was found that most of the DDT was absorbed on the surface of the suspended solids in the sample. Extraction with a mixed solvent of ether and *n*-hexane was used to remove DDT from the particles. The use of electrodialysis for determining the nature of organic substances in river water was reported by Ponomareva and Ettinger (*178*).

Pomeroy (*177*) pointed out that floating oil on a stream has a different significance from the colloidally dispersed oil and therefore it should be determined separately. The author devised a test using a tall tube for flotation separation of the oil. Beaudoin (*14*) presented a simplified oil determination method.

The determination of surface active agents in water was investigated by Lewis and Herndon (*126*). The turbidimetric and the two-phase titration systems were studied, with preference given to the latter. The procedure for the determination of an anionic detergent involves the addition of 1 part of chloroform to 3 parts of sample, and the use of an acid dye such as Pontamine Fast Red 8 BNL. The sample is titrated with a cationic agent such as cetyl trimethylammonium bromide to the point of first transfer of color to the organic phase.

DISSOLVED OXYGEN

Widespread interest in the instrumental determination of dissolved oxygen is apparent, and the advantages of the instrumentation are considered in several papers. Ippen and Carver (*103*) compared two polarographic methods, the dropping mercury electrode and the rotating platinum electrode, and concluded that the latter instrument possessed desirable characteristics in freedom from air bubble interference, presentation of a linear record, and superior ruggedness. Busch and Sawyer have presented the results of a study of the use of the dropping mercury electrode instrument in two papers (*27*, *28*). The polarographic method was applied to the determination of oxygen in radioactive wastes, as the exposure of personnel to activity is less than in the Winkler method and time requirements are less. The authors found that potassium chloride could not be used as a suppressor because it reduced the solubility of oxygen. A single potential technique which eliminates residual current effects was used. Good checks with the Winkler method were obtained. A report on the use of the Pauling magnetic oxygen analyzer in studies of oxygen uptake was given by Hoover, Jasewicz, and Porges (*94*).

Chemical investigations of the dissolved oxygen determination cover a wide range with reports involving the oxidation of indigo carmine, the acid chromous reagent, and the bromine difference method as well as modifications of the standard Winkler method. Loomis (*129*) has presented a colorimetric micromethod based on the reaction of oxygen with reduced indigo carmine to give a color. The reaction is carried out in a tuberculin syringe using only a 0.50-ml. sample and the color is read in a spectrophotometer. A linear relationship exists at 580 mμ. Stone and Sigal (*218*) modified the acid chromous titration previously reported by Stone and Eichelberger to eliminate nitrite interference. Oxygen is determined by difference using two samples, one of which is boiled to remove oxygen. Wickert and Ipach (*239*, *240*) studied the bromine difference method and presented modifications which permit application of the test in the presence of strong reducing agents. Freier (*52, 53*) reported two studies of the dissolved oxygen determination with *o*-tolidine and *o*-toluidine. Harper (*78*) studied a semimicro modification of the Winkler procedure. Verbestel, Berger, and Royer (*232*) described the preparation of oxygen-free water and reagents and construction of special apparatus for research in the dissolved oxygen determination. Zehender and Stumm (*248*) reported the determination of ozone in drinking water with *o*-tolidine.

BIOCHEMICAL OXYGEN DEMAND AND CHEMICAL OXYGEN DEMAND

A critical study of procedures for the derivation of B.O.D. velocity constants was conducted by Ludzack, Moore, and Ruchhoft (*136*), who tested the moments method of Moore over a wide range of rate constants. The k and L values so obtained gave good agreement with the constants derived by the standard Reid-Theriault method. Agreement of the Thomas slope method with the Reid-Theriault values was less satisfactory. Simplification of the standard B.O.D. test was attempted by Orford, Rand, and Gellman (*170*), who studied a modified single dilution technique denominated "jug dilution." Two 1-gallon samples are used. One of the bottles is used to supply the samples for analysis and the second bottle is reserved for refilling the sample bottle to the mark after each withdrawal. The sample withdrawals must be small in relation to the total volume. It is claimed that the jug dilution technique gives smoother B.O.D. curves and considerably more operating convenience than the conventional procedures. Radioactivity tracer techniques were applied to the study of biological sewage oxidation by Rorschach and Reid (*192*), who studied the phosphorus-32 uptake of slime and calculated a reaction coefficient. The value of k agreed with that found under other conditions.

The work of evaluating the C.O.D. and oxygen consumed tests has been continued by several investigators. Roberts and Sanderson (*189*) compared the U. S. Public Health Service dichromate procedure against the standard permanganate. The conclusions agree with the findings of previous workers that the dichromate method affords superior reproducibility and greater oxidation for highly polluted samples. Silver catalyst improves the oxidation. Popinsky, Porges, and Hoover (*179*) have made a comprehensive report on the application and significance of the C.O.D. test. Application to wastes from dairies, refineries, and soap plants were considered. Ingols and Kirkpatrick (*99*) used three oxygen demand tests in a study of chromium toxicity: the dilution B.O.D., the procedure of Gelman and Heukelekian with Sierp apparatus, and the methylene blue procedure by Ingols. Czensny (*39*) studied correlation between the B.O.D. test and permanganate consumption. Hermanowicz and Jasinska (*84*) evaluated the oxidizing ability of several C.O.D. systems, including hypobromite, against various types of organics. In a paper summarizing the methods of analysis suitable for trade wastes Ettinger (*47*) discussed the relationship between the C.O.D and B.O.D. tests and pointed out some advantages of the former. In a study of the analysis of packinghouse wastes Hirlinger and Gross (*89*) evaluated the dichromate reflux method for C.O.D. and concluded that it was the most reliable of the currently available methods. An attempt was made by Niedercorn, Kaufman, and Senn (*166*) to devise an oxygen consumed determination not subject to chloride interference based on the permanganate oxidation system. It is stated that the method gives essentially comparative results.

BORON AND SELENIUM

The high neutron absorption of boron makes it necessary to maintain boron at extremely low levels in reactor water. Consequently, the analytical methods for the determination of boron in reactor water must be highly sensitive. The conventional colorimetric methods fail in this respect. Fizzotti and Selmi (*49*) developed a colorimetric method for reactor water analysis based on the color change of an acid-base indicator in a borate solution to which mannitol has been added. Gorfinckel and Pollard (*64*) have presented modifications of the 1,1-dianthrimide determination of boron. While the work was done in connection with plant and soil analysis, it is likely that water chemists who use the dianthrimide method in their work will be interested in these authors' findings. Martin and Mais (*142*) studied several boron determinations in which the colorimetric reagent was made up in concentrated sulfuric acid and they reported that glacial acetic acid could be substituted with a consequent improvement in the determination.

The catalytic acceleration effect of selenium on the bleaching of methylene blue by sulfide was used by Goto, Hirayama, and Ikeda (*66*) for a quantitative determination of selenium. Selenium concentrations as low as 2 γ could be detected. Interference was encountered with several heavy metals. Selenium reacts with cyanide ion to form a stable anion which can be brominated. The resulting cyanogen bromide reacts with iodide to give free iodine. This reaction series was made the basis of a quantitative selenium determination by Schulek and Koros (*201*). Rudra and Rudra (*195*) used reduction with ascorbic acid to give a brown turbidity as a colorimetric determination, and stated that the test is sensitive to 1 p.p.m. of selenium.

RADIOACTIVITY

The inherent limitations of the end-window counter in the assay of beta activity was discussed by Setter, Nader, and Hagee (*203*) in a paper wherein the evaluation of back-scatter, absorption, and geometry effects for an end-window counter were considered. The authors' conclusion was that the lower limit of detection for the end-window counter was on the order of 10^{-7} μc. per ml. Considerably improved performance was obtained with the use of an internal counter. The evaluation of such an instrument was described in a report by Nader, Hagee, and Setter (*159*), in which the determination of the factors necessary for the conversion of counting rates to absolute activities was considered. Performance curves for phosphorus-32, thallium-204, and sulfur-35 are given. In an earlier paper by Nader, Setter, and Goldin (*160*), the preparation of samples for counting of both the dissolved and the particulate phases of a water sample was considered.

Loosemore (*130*) has considered the monitoring of water supplies for fission product contamination by means of a "skirt" type of liquid counter. This is a type of Geiger tube in which the sample is poured into an annular well which surrounds a thin-walled counting tube. The use of this device was considered to be the practical solution to the problem of maintaining a reproducible geometry under conditions of field use by relatively unskilled personnel. A liquid counter useful in the range of 5×10^{-4} to 0.5 μc per ml. was designed. A calibration device is described. The comparative merits of phosphorus-32 and a mixture of strontium-89 and cerium-144 were considered. Lacy and Kahn (*121*) reviewed three portable Geiger counters, and one model of an electroscope for radioactivity monitoring in water. The instruments were considered satisfactory for the determination of radioactivity at the levels set as tolerance limits by the National Bureau of Standards (*163*). Another National Bureau of Standards publication (*162*) has covered radiation hazards, detectors, measuring instruments, and other phases of methods and procedures, including water-sampling techniques for alpha and beta assay.

The field of the determination of natural radioactivity background was reviewed by Healy (*81*), who included a section on water. Radium, thorium, and uranium are the three important elements, and their approximate concentration ranges in several types of natural waters are tabulated. Hursh (*98*) carried out the determination of radium in 41 public supplies. The radon emanation method was used with 20-liter samples to attain the desired sensitivity of 10^{-14} grams per liter. Hydrochloric acid was added to the samples to minimize absorption losses. The radium content of the supplies investigated ranged from a low of 0.02×10^{-13} to a high of 65×10^{-13} grams per liter for some deep well samples. The assay of beta activity in water supplies with an end-window Geiger tube was reported by Wheler, Kaufman, and Eliassen (*236*). The self-absorption correction for several energy levels was evaluated. Damon and Kuroda (*40*) measured the activity of rainfall at Fayetteville, Ark. Saito (*197*) studied the distribution of radium and thorium in Japanese river waters and the Pacific Ocean. It was found that the two metals show very different propensities to form radiocolloids, with thorium showing a readiness to be absorbed by even relatively large particles. Radium does not readily form radiocolloids. Therefore it is concluded that most of the thorium will deposit in the river or coastal waters and the radium will be in excess in ocean waters.

Interest has always been shown in the determination of the radioactivity of mineral and hot springs, and there is no abatement at the present time. Rosenblatt and Lindeman (*193*) studied the activity of the hot springs of Tiberias and found the major activity due to radon. Kuroda and Yokoyama (*120*) used the fontactoscope to determine thoron in mineral waters by following the decay rate. Umemoto (*227*) developed a procedure for the determination of radium in mineral springs using dithizone with lead and bismuth as a carrier for concentration and separation. Kimura, Kuroda, and Yokoyama (*112*) studied the equilibrium between radon and its decay products using the electroscope. Kuroda, Damon, and Hyde (*119*) determined the activity of some of the springs of Hot Springs National Park.

The problems of continuously monitoring a large stream contaminated with traces of artificial activity were thoroughly discussed by Foster and Rostenbach (*50*). The problem requires consideration of the entire flora and fauna pattern of the stream as well as the water and suspended sediment phase and representative methods of sampling as well as valid analytical methods have to be developed for each component of the stream. The authors reviewed the Columbia River program, and described the methods used in detail. Kochtitzky and Placak (*114*) considered a similar subject in an article entitled "How to Survey a Stream for Radioactive Substances." A report on the study of radioactive fallout was made by Thomas, Kleinschmidt, Parker, and Bell (*224*), who surveyed Massachusetts surface waters following a series of nuclear weapon experiments in Nevada. A noticeable increase in activity was observed.

The necessity for a reference standard for correlation of radioactivity results between different investigators is becoming more apparent. Wheler, Reynolds, and Brooksbank (*237*) have recommended thallium-204 as a standard for beta activity measurements of fission products. The energy level of 0.78 m.e.v. is reasonably close to the average level of 0.95 m.e.v. for a fission mixture several days old. Gerstein (*59*) has reviewed the progress of the American Water Works Association Task Group on radioactivity. A paper which is not directly concerned with the determination of radioactivity in water, but nevertheless presents much analytical information applicable to the field of water analysis, is that of Hudgens (*96*).

URANIUM AND THORIUM

Grimaldi, May, Fletcher, and Titcomb (*70*) have published a bulletin covering the methods for analysis of uranium and thorium developed in the United States Geological Survey. Two fluorimetric procedures for the analysis of uranium in fresh and saline waters are presented, the "dilution" or direct method and the extraction method. The latter procedure involves isolation of the uranium in a mixture of ethyl acetate and nitric acid prior to performing the fluorimetric determination. A sodium fluoride–carbonate flux was used. A previous publication (*69*) gives further experimental details for both uranium methods. The design of fluorimeters is treated in detail in the two papers. Price, Ferretti, and Schwartz (*182*) reported on the determination of uranium by fluorimetry using the "dilution" technique. Pure sodium fluoride was used as the flux and "spiking" of samples with known uranium was used to eliminate the effects of "quenching" metals. It is stated that uranium can be determined down to concentrations as low as 10^{-10} gram using the recommended procedure. The uranium content of sea water off the coast of Japan was determined by Nakanishi (*161*) with the fluorimetric method and was found to average 2.8 γ per liter at a depth of 4000 meters. This level is somewhat higher than previously reported.

Several papers not confined specifically to the determination of uranium in natural waters have presented data of interest to anyone concerned with this problem. A round table discussion of fluorimetric analysis under the chairmanship of C. E. White (*238*) gives a review of the subject with some comment on uranium. Glover (*62*) described the concentration of uranium prior to fluorimetric analysis by means of gathering with protein. Seyfang and Smales (*204*) reported on the determination of uranium-235 in mixtures of naturally occurring uranium isotopes by radioactivation.

ISOTOPIC ANALYSIS

The determination of deuterium oxide in water samples was performed by Washburn, Berry, and Hall (*234*) with a mass spectrometer system wherein the necessity for the preliminary conversion of water into a gaseous compound was bypassed. Either a 180° or 158° mass spectrometer could be used with the ion source in a strong magnetic field and about five times normal voltage applied to the ion repelling electrode. By this means it was possible to minimize the interference of an ion of mass 19 (believed to be H_3O^+) and determinations to differences as low as 0.0006 atom % could be made at low concentrations. Haldeman (*76*) used the variation in resistance of a hot filament with the vapor pressure of a condensed vapor phase as a measure of the deuterium oxide content. Construction of the hot wire cell and the assembly of the resistivity measuring circuit are described in the paper. The agreement between mean determinations and the known values of standards was 0.3%. Friedman (*54*) used the mass spectrometric method to determine the deuterium oxide content of a number of water samples. The ratio for ocean waters was found to range from 0.0153 to 0.156 mole % and for fresh waters from 0.0133 to 0.0154 mole %. The higher deuterium oxide concentration of ocean waters is thought to be due to fractionization by evaporation. Baertschi (*11*) studied the distribution of H_2O^{18} in natural waters by means of the mass spectrometer. Rain water, surface water, ground water, and water from minerals were studied. Variations in isotopic content ranging between −7.1 and +18% from the norm were discovered and are discussed in relation to the nature of the source. Chinard and Enns (*33*) reported studies on a method for rapid reduction of samples with zinc dust preparatory to mass spectrometer analysis.

Jenkins (*108*) reported on the determination of tritium in water. The sample is allowed to stand with 1 or 2 grams of ammonium chloride for 10 minutes, which apparently is sufficient time for exchange equilibrium to be reached. The water is distilled off and the salt residue is ground up and analyzed for beta activity in a windowless flow counter using an infinitely thick sample. Grenon and Viallard (*67*) described the determination of tritium using an external cathode counter of the Maze type.

MISCELLANEOUS

Calmon (*30*) has presented a review of the application of ion exchange methods to water analysis. A discussion of theory is followed by a consideration of applications to boiler water analysis and cooling water analysis including the determination of dissolved solids, total cation content, dissolved oxygen, heavy metals, and fluoride. Calmon (*29*) has also described the determination of the total dissolved solids in water by measuring the volume change of a resin caused by the take-up of ions. Rath (*185*) has considered the application of ion exchange in the analysis of boiler water.

A study of the scattering of light in natural waters was presented by Atkins and Poole (*9*). The forward and back scatter are approximately the same for distilled water, and a change in the ratio indicates the presence of turbidity. In sea water, three quarters of the scatter could be removed by filtration through a membrane with a 1-micron pore size. The light-scattering effect of surface and coastal waters was greater than that for the deeper waters. A polarographic determination of colloids in hot spring waters was carried out by Valdiguié and Garcia-Fernandez (*228*). The reducing effect of the colloidal matter on the polarographic maximum of oxygen was measured. A correlation with the chemical determination of silica was observed. Sainclivier and Berthois (*196*) determined suspended sediments by absorption on diatomaceous earth. Valin (*229*) reviewed methods for the determination of color in water.

A modification of the evaporation procedure for the determination of the total salt content of sea water was presented by Guntz and Kocher (*73*), who added sodium fluoride to transpose the alkaline earth salts to fluorides and thus minimize evaporation losses. Kruse (*118*) described the preparation of membrane filters of specific porosity for various uses in water analysis. Matida (*143*) reviewed the methods available for the determination of total phosphorus in water with consideration of the sources of the several forms. Bradwell (*23*) discussed the analytical aspects of determining sea water infiltration, and considered the use of the ratio of chloride to bromide.

Heide (*83*) has discussed the geochemistry of fresh waters which, although not strictly analytical in nature, will be of interest to water chemists. Problems discussed include sampling, nonhomogeneity of streams, changes in composition of samples on standing, and elements present as colloidal dispersions or adsorbed on larger particles.

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