ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Change of Name Approved

THE proposal to change the name of the Division of Analytical and Micro Chemistry to the Division of Analytical Chemistry has been approved by an overwhelming vote of the division members.

We believe the change is a highly desirable one. Certainly it does not mean any reduction in the attention paid the field of microchemistry by either the division or this publication. It does provide a more all-inclusive name for the division and an opportunity to serve better the needs of analysts in all branches of analytical chemistry.

We are now presented with a great challenge—to build the division into the largest, most active, and most effective division within the Society. The groundwork has been laid this year under the able leadership of Wayne A. Kirklin. Still further gains will be achieved in 1950 when Grant T. Wernimont takes over the chairmanship. What is needed are interested analysts in each analytical laboratory who will solicit division memberships. In size there are strength and the possibility of increased service to the profession of analytical chemistry.

The Union Meeting at Amsterdam

LARGELY as the result of a proposal by I. M. Kolthoff at the 1947 meeting of the International Union of Pure Chemistry held in London that the union undertake the standardization of physicochemical data used in analytical chemistry, a decision was reached at the recent Amsterdam meeting to organize under the union a Section of Analytical Chemistry.

Heretofore the union has had only one commission dealing specifically with analytical chemistry—New Analytical Reactions and Reagents. The desirability of the step taken in Amsterdam need not be elaborated upon before an audience of analytical chemists.

The new section will be governed by a board of twelve, which will have authority to appoint committees to deal with specific problems and which is also authorized to offer its services in the organization of international meetings on analytical chemistry.

There is at present no analytical chemist among the vice presidents of the union and for this reason Sir Ian Heilbron has been placed in nominal charge of this section, but C. J. Van Nieuwenburg of the Netherlands will act as the director of the section until the time of the 1951 meeting. Professor Kolthoff is vice president, Professor Wenger of Geneva is the European secretary, and Dr. Ashley of Pittsfield (General Electric), is the American secretary.

Certainly the establishment of a Section of Analytical Chemistry will be looked upon as a very progressive step by analysts the world over and should encourage analytical chemists to support the 1951 meeting to be held in New York City.

Most heartening were the informal remarks of Edward Wichers, chief of the Chemistry Division, National Bureau of Standards, and a delegate to the Amsterdam meeting, speaking before the luncheon meeting of the advisory boards of ANALYTICAL CHEMISTRY and *Industrial and Engineering Chemistry* in Atlantic City last month. Real solid progress was made at the union meeting in Amsterdam and the outlook for the success of the union never was better, according to Dr. Wichers.

Women as Analysts

CORNELIA T. Snell of Foster D. Snell, Inc., speaking before the Division of Chemical Education in Atlantic City last month, described the expanding opportunities for women in the field of analytical chemistry.

The distaff side of the chemical profession compiled an enviable record during World War II, particularly in analytical work. It is encouraging to hear from Dr. Snell that representatives from a number of concerns now visit the placement bureaus of some of the women's colleges, looking for new talent.

The erroneous idea that chemical analysts (and we do not mean technicians) are somehow inferior to chemists in other branches of the science of chemistry has disappeared to a considerable extent. As a result of this trend in the thinking of the profession as a whole, young men and women are viewing a career in analytical chemistry with more favor than in the past.

If we accept Dr. Snell's description of analytical chemistry—"It is the cornerstone of all chemistry, whether research or some other seemingly unrelated aspect of science"—then there is no necessity for any one, man or woman, to view a career in analytical work with misgiving, provided, of course, the candidate possesses the many necessary qualifications.

Recording Infrared Spectrometer for Continuous Analysis

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AND
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A Perkin-Elmer recording infrared spectrometer has been adapted for use in the continuous determination of six different components in a flowing stream of sample. The percentage absorption at each of six key absorption wave lengths is automatically recorded every 5.75 minutes, using the energy at six selected reference wave lengths to determine I_0. The instrument is well stabilized, as the readings are compensated for the effects of changes in temperature, source intensity, slit width, detector sensitivity, amplifier gain, and dirt on the windows. Compensation is effective for variables producing energy changes as great as sixteenfold. Long-time stability of readings is ±0.5 to 1.0%.

THE applicability of infrared absorption spectrometry to industrial analytical problems has been well demonstrated by the number of papers on the subject in the past 10 years. Of these, the number dealing with quantitative analysis has been steadily increasing. Most of this work has been done on individual samples that were collected, stored, and subsequently measured, although there have also been several continuous-flow, continuous-analyzing instruments, particularly those of Schmick (*17*), Pfund (*15, 16*), Luft (*13*), Baird (*1*), Wright (*19*), Fastie (*6*), and Brady (*3*). All of these differ radically from the manually operated monochromators in that they use filters to isolate light of a wave length suitable for the determination of only one component.

Selective detectors, differential detectors, and selective sources have all been used to make energy measurements at selected wave lengths without spectral dispersion. For some simple analyses no spectral isolation of any kind has been used. By avoiding the use of a monochromator, relatively large amounts of radiant energy are available for measurement, so that good stability is easily obtainable. When denser filters are used to give greater spectral selectivity, light choppers and alternating current detection methods are used to stabilize the zero point. Balanced systems employing two separate beams of light from the source have been used in most cases to reduce the effects of source intensity variation.

These instruments have proved applicable to problems where isolated absorption bands exist for the unknown compounds in the short wave-length part of the spectrum. For more complicated cases where the absorption bands in the high energy part of the spectrum are all overlapping, the results are not so useful. As the wave length isolated by a given instrument cannot be readily changed, only one compound can be determined with one instrument and there can be no correction for overlapping absorption bands without using as many instruments as there are interfering components. For good control of most chemical processes the concentrations of several components must be known. For example, in catalytic dehydrogenation of butenes it is essential to know not only the amount of butadiene produced but also how much feed stock was consumed in the process. This requires knowledge of two concentrations if the feed is a pure compound and of many more if it is not, and the composition of the unreacted material is important. To overcome this difficulty and to develop a method capable of using the absorption bands in the long wave-length, low-energy part of the spectrum, a prism spectrograph has been adapted to the continuous automatic recording of transmittances at a number of different wave lengths in the infrared region. This makes it possible to follow the concentrations of several components in a flowing stream and to correct for interferences between the different spectra by any of the commonly used methods (*4, 5, 7, 9, 10, 14, 18*).

PRINCIPLE

The measuring principle is a modification of that described by Heigl *et al.* (*9*), in which a "base line density" calculated from differences in the sample's transmittance at different wave lengths is used. In the new instrument a reference wave length is selected for every key absorption wave length where a transmittance measurement is desired. The reference wave length is selected to be as free as possible from absorption bands in the sample, or if desired, to coincide with other absorption bands of the interfering components to neutralize the effects of the interference.

In operation the signal corresponding to the radiant energy at a reference wave length is measured first and stored in the instrument while the energy at the corresponding key absorption wave length is being measured and divided by the reference energy, and the ratio recorded. The instrument then advances to the next reference wave length, erases the stored information, and repeats the cycle. The signal received at each wave length is independently adjustable, so that energy differences between pairs of wave lengths may be corrected. If there is more or less energy at the reference wave lengths than at the corresponding measuring wave length, the resulting signal may be reduced or increased to make the two equal in the absence of sample. The instrument then reads directly in per cent transmittance at the key wave length; otherwise it reads in transmittance multiplied by an adjustable factor. In this way the scale can be expanded to make very strong absorption bands more easily readable.

This measuring principle has the advantage of compensation for extraneous variations. For example, if dirt or fog accumulates on the windows of the absorption cell, practically the same amount of light is removed by it at both the reference and the absorption wave lengths, and the ratio of the two intensities is almost entirely unaffected. Similarly, if the amplifier gain changes slowly, its effect is removed; and if the sources temperature changes, the compensation is good over short wave-length intervals.

DESCRIPTION

A block diagram of the continuous recording infrared spectrometer and accessories is shown in Figure 1.

Light from the source is interrupted seven times a second by the rotating shutter to give a pulsating light beam suitable for detection by alternating current methods. It passes through a continuous flow sample cell and a monochromator to a detector, where it is converted to an electrical signal proportional to the light intensity. After amplification the signal is rectified, filtered,

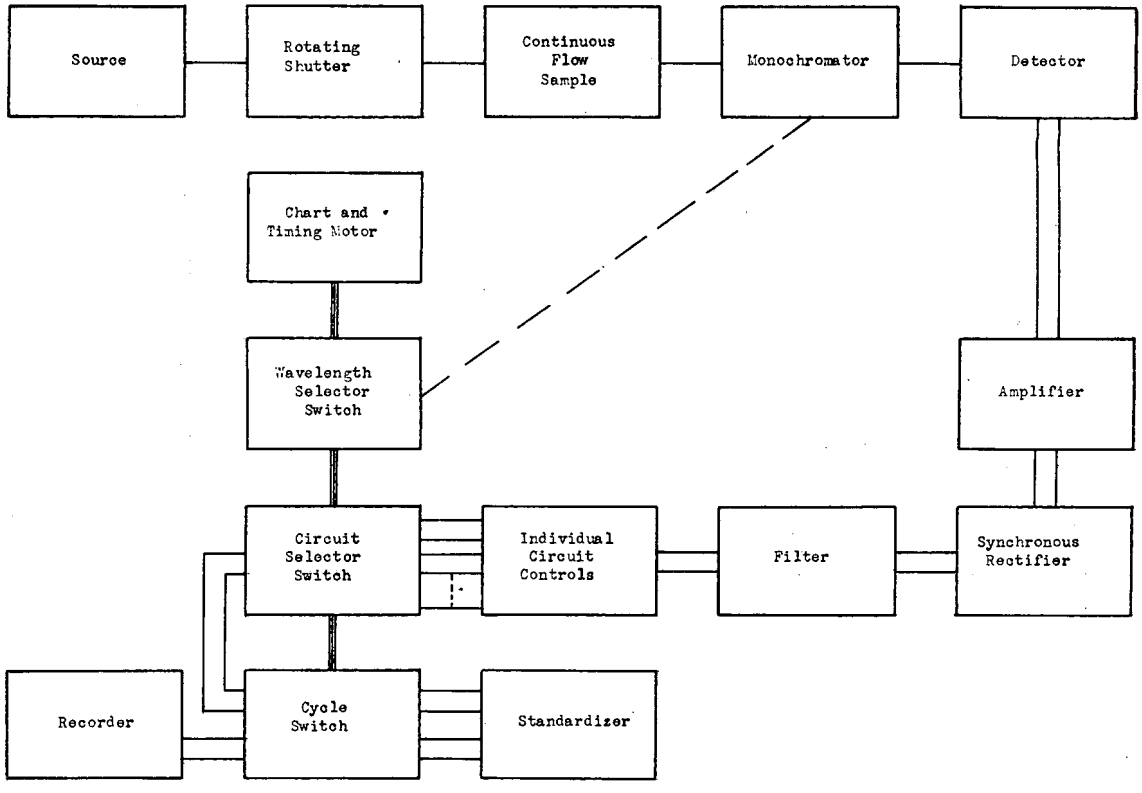

Figure 1. Block Diagram

and split into twelve parallel circuits with a separate rheostat control in each. All the circuits go to a circuit selector switch that picks out each successively. A main timing motor simultaneously drives the circuit selector switch, the wave-length selector switch, and the cycle switch so that the circuit selected is always the proper one for the wave length isolated by the monochromator.

Following the circuit selector switch is the cycle switch that connects the signal alternately to the standardizer and to the recorder. The standardizer contains the memory device that stores the energy measurement at each reference wave length for comparison with that at the following absorption wave length. It automatically attenuates the signal at each reference wave length by the right amount to give an output of exactly 10 millivolts. This standardized signal is connected to the recorder at the end of each standardization cycle, and its value is printed to show that the operation was completed. The attenuation is maintained for the succeeding absorption wave length while the output is being measured and recorded. At the next reference wave length the recorder is again disconnected, the attenuation readjusted to give the standard signal, and the cycle repeated.

The operation of this cycle is illustrated in Figure 2, which shows a schematic section of the recorder chart with the operation performed by each part of the circuit at each time marked beside it. There are twelve wave lengths, six for reference and six for absorption measurements; 28.8 seconds are required at each wave length, or a total of 5.76 minutes for a complete group of measurements. The group is automatically repeated at this frequency.

The monochromator is a standard Perkin-Elmer Model 12-C similar to the one described by Barnes *et al.* (*2*) and Gore *et al.* (*8*) with the substitution of a lithium fluoride prism for the sodium chloride prism and the addition of a motor-driven turret for automatic wave-length selection. Figure 3 is a schematic view of the additional parts, illustrating their operation.

When the motor runs, the slotted bar on the cam pulls the Littrow lever off the stop and releases the turret so that it is free to turn. The cam on the end of the motor shaft advances the ratchet on the turret one notch and then lets a spring pull around. Finally, the Littrow lever is let back on the new stop after it has been locked in position by the pin. The microswitch operated by the same cam stops the motor at a fixed position when the cycle is complete. Coil springs not shown in the figure supply the force to advance the turret and lock it in position. Leaf springs hold and act as bearings for the cam follower and turret locking pin.

The detection and amplification system is essentially that described by Liston (*11*) and used as standard in the

Time, Minutes	Chart 0 25 50 75 100	Wave Length	Cycle Switch to	Standardizer	Recorder
		Reference	Standardizer	Adjust to standard	Disconnected
	1.		Recorder	Fixed	Print standard value
1	2.	Absorption	Recorder	Fixed	Print transmission
		Reference	Standardizer	Adjust to standard	Disconnected
	3.		Recorder	Fixed	Print standard value
2	4.	Absorption	Recorder	Fixed	Print transmission
		Reference	Standardizer	Adjust to standard	Disconnected
	5.		Recorder	Fixed	Print standard value
3	6.	Absorption	Recorder	Fixed	Print transmission
		Reference	Standardizer	Adjust to standard	Disconnected
	7.		Recorder	Fixed	Print standard value
4	8.	Absorption	Recorder	Fixed	Print transmission
		Reference	Standardizer	Adjust to standard	Disconnected
	9.		Recorder	Fixed	Print standard value
5	1.0	Absorption	Recorder	Fixed	Print transmission
		Reference	Standardizer	Adjust to standard	Disconnected
	1.1		Recorder	Fixed	Print standard value
6	1.2	Absorption	Recorder	Fixed	Print transmission
		Reference	Standardizer	Adjust to standard	Disconnected
	1.		Recorder	Fixed	Print standard value
7	2.	...	...	....	....

Figure 2. Section of Recorder Chart

Showing station numbers printed on it and corresponding functions of units

Perkin-Elmer Model 12-C recording infrared spectrometer, modified to give greater output.

The detector is a thermocouple like the one described by Liston with sensitivity of 6 microvolts per microwatt, 90% response to light modulated at 5 cycles, and target area of 0.2 × 2 mm. The amplifier is the breaker type described by Liston *et al.* (*12*) with the substitution of a 6V6 tube for the 6N7 tube in the output stage to increase its useful output voltage.

Light from the source is interrupted at approximately 7 cycles per second by the light chopper. The radiation reaching the thermocouple produces in the thermocouple an alternating current potential of the same frequency. Because the hot junction of the thermocouple may be at a different temperature from the cold one, owing to ambient temperature variations, there may be present a direct current potential in addition to the alternating current one. Both potentials are fed into the input breakers of the amplifier, where they are converted into 75-cycle alternating current. The first stage of amplification is accomplished by the input transformer. The signal is then carried through three stages of a conventional resistance capacitance coupled alternating current amplifier and returned to the output breaker, where it is converted back to its original form (although pulsating and amplified several million times)—i.e., a 7-cycle alternating current potential and a direct current potential.

Figure 4 is a schematic wiring diagram showing how the circuit is connected.

The breaker amplifier output is separated into its direct and alternating current components by a resistance shunted with a 4000 μf. condenser and choke. The direct current component is fed back through a high resistance to the thermocouple circuit in the proper sense to oppose the direct current output of the thermocouple. In this way the effective gain of the amplifying system is made much lower for direct current signals than for alternating current ones. The signal is led to the rectifying breakers on the same shaft as the chopper shutter. Here the alternating current potential caused by the radiation from the globar is rectified and the residual direct current potential produced by the ambient temperature at the thermocouple is converted to alternating current. The principal output signal is now the rectified alternating current due to the modulated light. The filter following the final breakers smooths the pulsating output to a steady direct current and eliminates the alternating current component. A third set of

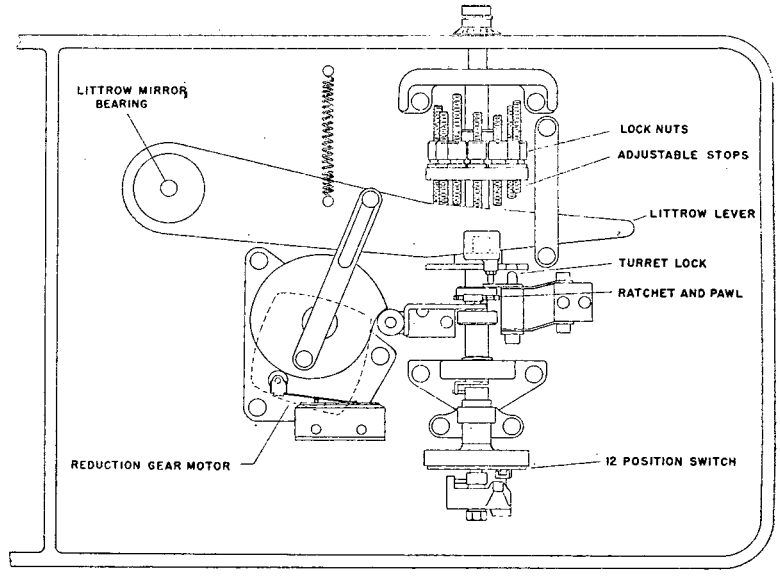

Figure 3. Bottom View of Right End of Spectrometer

Showing wave-length selector turret under monochromator

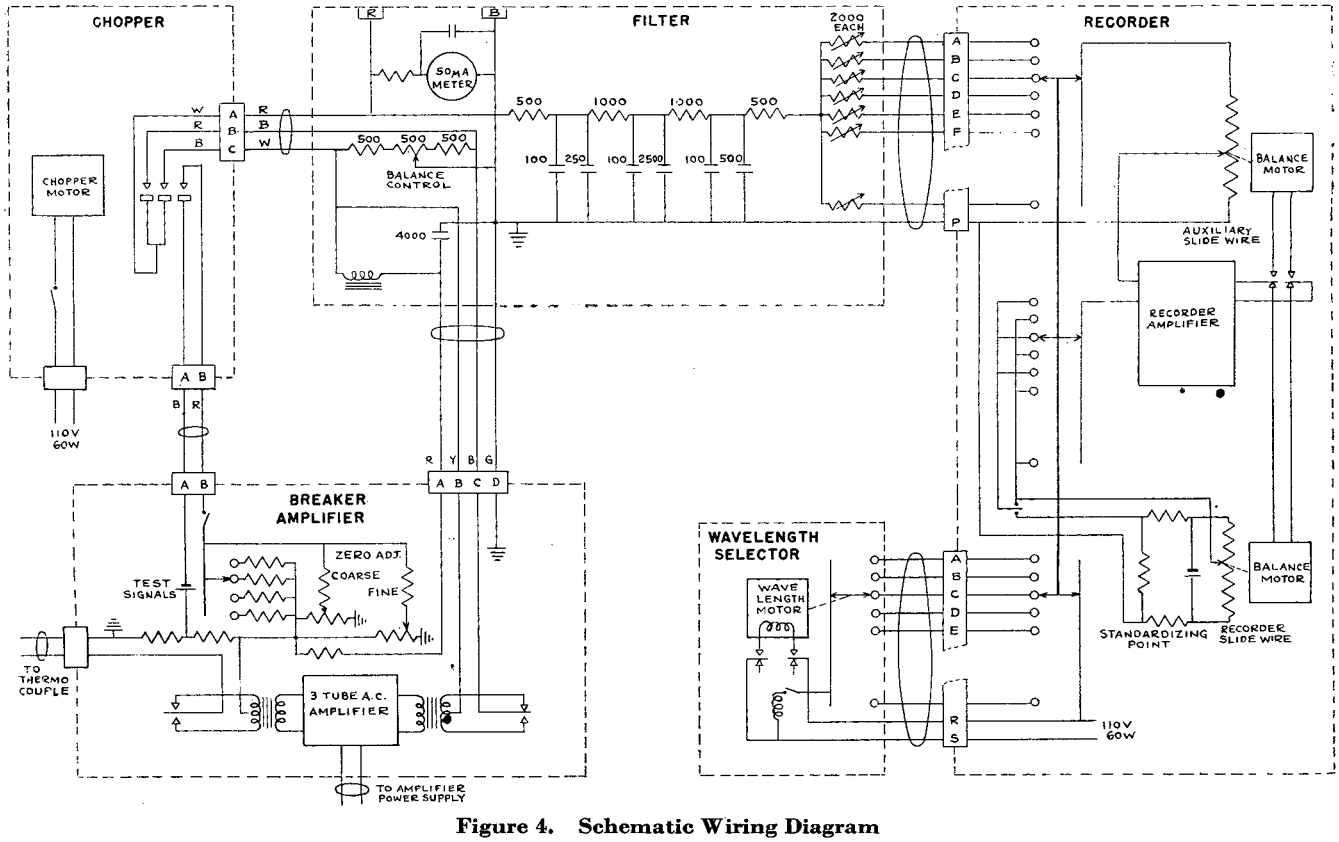

Figure 4. Schematic Wiring Diagram

Resistances in ohms, capacitances in microfarads

breakers on the chopper shaft modulates a test signal from the amplifier at the same frequency as the chopper.

The output filter is the same as that for standard Model 12-C scanning spectrometers, with the addition of a small output meter reading directly in rectified amplifier output and some larger condensers and resistors. The time constant is fixed between 3 and 4 seconds. The filtered signal is broken into twelve different circuits, each with a separate variable resistance to give independent control of the gain in each circuit. As only one circuit is used at a time, the presence of the others has no effect, and the gain settings are independent. Coordination and timing of the whole system are controlled by the chart motor in the Leeds & Northrup multipoint recorder, made especially for the purpose. It drives two double-pole, twelve-station switches. One controls the wave length turret in the monochromator, the other the selection of the output circuit and its connection to the standardizer or recorder. Half of one switch is not used. When the wavelength turret is set at even-numbered reference stations, the filtered signal is connected across a slide-wire potentiometer that attenuates it by an adjustable amount. An auxiliary motor like the main slide wire motor automatically adjusts the potentiometer to give a fixed ouput on each reference station. When the wave length advances to the following absorption station, the potentiometer is left in this position, and the signal from it is balanced against the regular recorder slide wire and recorded.

The wave-length turret in the monochromator has another twelve-station switch mounted on its shaft. Each station is connected to the corresponding master synchronizing station in the recorder, so that power flows through the two switches only when both are set on the same station. This power operates a relay that opens the turret motor circuit and stops the motor. When the master switch advances, the control circuit is broken, the relay closes, and the motor runs until the turret reaches the proper station and completes the control circuit to open the relay. An additional, normally open switch operated by the cam prevents the motor from stopping until the Littrow lever is in place on the new stop.

There is no provision for changing the slits simultaneously with the wave length, as it has been found that the standardizing mechanism has a wide enough range to permit making measurements over practially the whole range of the lithium fluoride prism at one slit setting. There is, however, a possibility of adding such a mechanism if the instrument is to be used with a different prism over a wider energy range. The unused half of the twelve-station switch in the recorder is connected to a plug and available for synchronizing slit changes with the rest of the operating cycle, either at every station or, preferably, only at the reference wave lengths.

PERFORMANCE

The important features of an infrared spectrometer for continuous, automatic use are stability, reproducibility, and reliability. Extreme precautions have been taken to obtain these properties. The use of a light chopper and synchronous rectifier practically eliminates the zero drift that is likely to cause trouble in infrared absorption instruments. The frequent standardization practically eliminates the effect of amplifier gain change or thermocouple sensitivity change and greatly reduces the effect of slow changes in the source temperature. Rapid source changes are prevented by the regulated power supply. Effects of temperature change on the wave-length calibration scale are reduced by automatic compensation for changes in the prism's refractive index. The instrument has been operated intermittently for times up to 6 days over a considerable period. The results of tests to determine its characteristics and to evaluate the effects of different variables are given in Figures 5 and 6 and Tables I to IV.

Table I gives the source stability measured as the peak to peak percentage variation in intensity in a period of 20 minutes. To get the best stability the globar was soldered into its contact electrodes with Hanovia silver paste solder. Table I shows that in the generally used part of the infrared region short-time source fluctuations are smaller than amplifier gain changes, and only near the visible do they become perceptible. Long-time changes due to aging of the globar are still present and must be corrected for by manually adjusting the power input.

Table I. Source Stability

(Expressed as maximum percentage variation in intensity in 20 minutes)

Power Supply	Wave Length, μ	Peak to Peak Variation, %
10-microvolt test signal	...	0.5
500-watt Raytheon constant-voltage transformer	4	0.75
Transformer and 3 iron wire ballast lamps	4	0.5
Transformer	0.8	2.0
Transformer and 3 ballast lamps	0.8	0.75

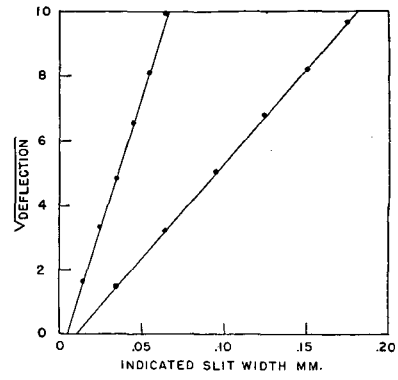

Figure 5. Linearity

Figure 5 shows the slit performance at two sensitivity levels. Recorder deflections were measured over a wide range of slit widths, and the square root of deflection was plotted against slit width. If the slits are reproducible and everything is properly lined up, deflection should vary as the square of the slit width, to give straight lines in the figure. Slit accuracy and amplifier linearity are indicated by the consistency of the points and the straightness of the lines, respectively. If the slits did not open and close simultaneously, the lower ends of the lines would curve downward more steeply, indicating that the intensity had gone to zero sooner than predicted from the measurements at wide slits. This would be especially noticeable in the higher sensitivity curve. If the output stages of the amplifier were nonlinear, the points would fall along a curve rather than a straight line, and both lines would show the same curvature. The fact that the two lines do not go to zero intensity at the same slit width is real but as yet unexplained.

Table II. Compensation for Amplifier Gain Change by Standardization

(Expressed as recorder deflections at wave lengths near 4.3μ CO_2 band, with reference stations all set at 2630 cm.$^{-1}$ Sensitivity is input signal required to give recorder full-scale deflection at 2630 cm.$^{-1}$ after standardization. Amplifier gain is expressed as its filtered output signal in millivolts for 6 microvolts' input)

Amplifier Gain	Sensitivity for Full-Scale Recorder Deflection, μv.	Readings at Frequencies, Cm.$^{-1}$ 2300	2330	2364	2630	12,000
160	6	30.5	8.8	5	90.5	0
80	6	31	10	6	90.2	1
40	6	32	11	6.8	90.5	2.0
20	6	34.8	12.8	8.2	90	2.8
10	5.5	36	13.8	9	90.8	2.5

The effectiveness of the standardizer in correcting for changes in amplifier gain and source intensity is shown in Tables II and III. Table II shows that a sixteenfold change in amplifier gain changed the recorder readings 4 to 6 divisions at small energy values and practically none at large values. There was also a 2.8-division change in the zero point as measured at 12,000 cm.$^{-1}$ where the intensity was negligible. This is probably caused by the chart zero's not coinciding with the recorder's electrical zero. Table III shows that the effect of changing source temperature is

also corrected for over a threefold range of power input. There is very little change for wave lengths close to the reference wave length and progressively more as the points depart from it. The change of zero previously observed is now absent except for a small effect at the brightest setting, due to perceptible radiation at this point. At the reference frequency the change in recorder reading was only 0.5 division; 330 cm.$^{-1}$ away it was 11.5 divisions, or 18%. The corresponding change of intensity at the reference point was a factor of 2.6, giving a compensation factor of 14.

The effect of varying slit width is shown in Table IV. Compensation at the reference wave length is again excellent, and at other points it appears to vary slightly, depending on the sharpness of the absorption band at the point. There appears to be a change of 3 divisions in the recorded zero point for a 3.3-fold change in slit width. The energy change corresponding to this slit change is slightly more than 10. The readings at the three points in the carbon dioxide absorption change by 6 to 7 divisions. At the narrow 3337 cm.$^{-1}$ ammonia band the reading increased 8.5 divisions as the slit width was increased. Then it decreased again, indicating that the linear range of the amplifier was exceeded at the extreme slit width.

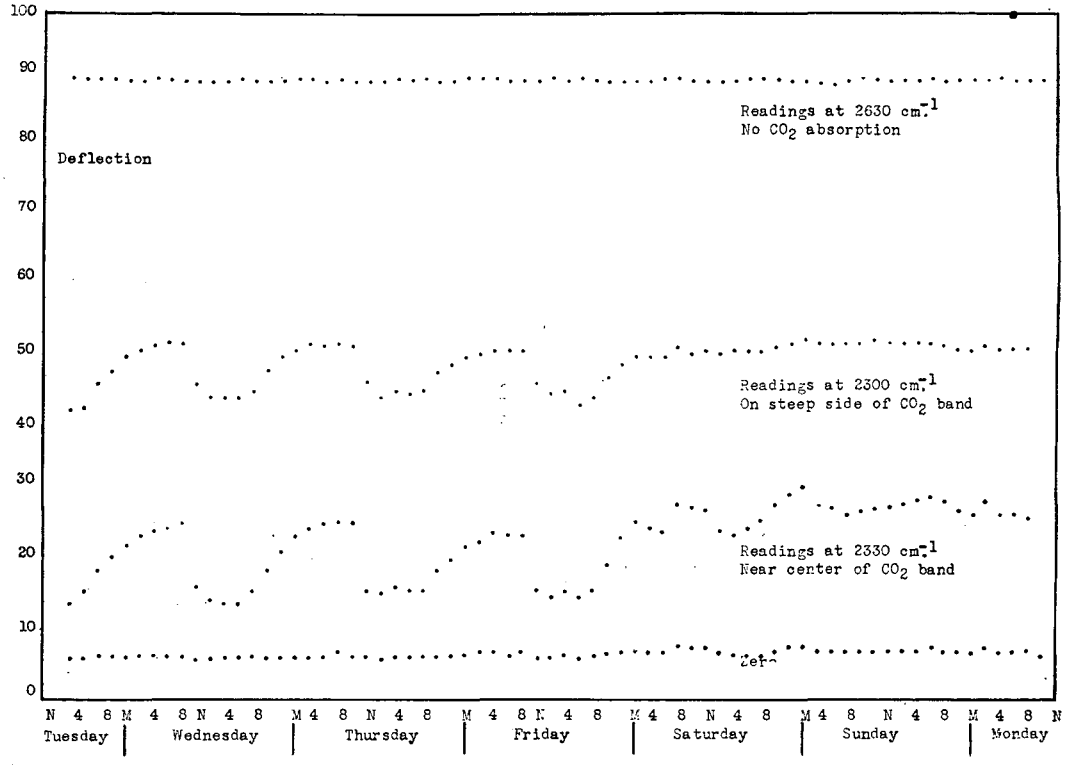

Figure 6. Carbon Dioxide Absorption in Atmosphere of Perkin-Elmer Plant
Working hours, 8 to 12 and 1 to 5; Saturday irregular; Sunday none

Table III. Compensation for Source Temperature Change by Standardization

(Expressed as recorder deflections for different power input. Reference stations were all set at 2645 cm.$^{-1}$)

Power to Globar, Watts	Amplifier Output at 2645 Cm.$^{-1}$, Mv.	Deflections at Frequencies, Cm.$^{-1}$ 2315	2345	2379	2645	3337	12,000
50	53	19	14	25	90.5	63.5	1
100	94	17.8	13.5	24	90	71.2	1
150	144	17.5	13.5	23.5	90	75	1.5

Table IV. Compensation for Slit Width Change by Standardization

(Reference stations were all set at 2645 cm.$^{-1}$)

Slit Width, Mm.	Amplifier Output at 2645 Cm.$^{-1}$, Mv.	Readings at Frequencies, Cm.$^{-1}$ 2315	2345	2379	2645	3337	12,000
0.055	16	16	13	20	91	63.5	91
0.095	53	19	15	23	90.5	66	90.5
0.145	131	19.5	16	23.5	90.5	71	90.5
0.165	163	20	16	24	90.5	71	90.5
0.195	>160	23	19	26.5	90	68	90

Wave-length reproducibility was tested by repeat readings of intensity for a point on the sloping side of the carbon dioxide band where a small change of wave length caused a very great change in intensity. Readings over a period of 6 days repeated within 3 divisions on the recorder, corresponding to a maximum shift of 1.5 cm.$^{-1}$, all readings being taken at the same carbon dioxide concentration. These observations were made in a quiet, air-conditioned building and indicate the best performance obtainable. Vibration or other unfavorable conditions might reduce it. The instrument is compensated for change in wave-length calibration due to change in prism index with temperature. This effect is small and hard to measure accurately with a lithium fluoride prism which has about one quarter the rate of change of refractive index with temperature that salt has. Using a sodium chloride prism it has been found that about 90% compensation is possible by turning the small mirror mounted on the prism table with a bimetallic strip. Use of the correct bimetal for lithium fluoride should make the effect negligible except in extreme temperature changes.

Figure 6 shows the instrument's over-all performance in a 6-

day run measuring the carbon dioxide absorption in the atmosphere of the Perkin-Elmer plant.

Values were read off the record at 2-hour intervals, and each plotted point was an average of four to six readings. Excluding a few times when dirt on a contact made the measurement fail, the maximum fluctuation in the readings in a 10-minute period was generally ±1% for the 2630 cm.$^{-1}$ curve and ±0.5% for the others. The reference point for each curve was 2630 cm.$^{-1}$ The top curve shows the stability in measuring energy at the reference point, the bottom one the zero stability measured as the energy at a wave length near the visible where the source had practically no emission. The two intermediate curves record variations in the atmospheric carbon dioxide content. The lower one is set to a wave length near the maximum absorption, the upper one at a point on the steeply sloping side of the band. Both curves show a sharp break when work started and a gradual recovery after quitting time, as well as the effects of a few irregular workers on Saturday and no work on Sunday. The effect of lunch hour appears in the 2 o'clock reading on two of the days. On the original records it was always pronounced at one o'clock. The correlation between these two curves indicates the wavelength reproducibility, as a small change in wave length would have changed the upper curve much more than the lower one.

In addition to indicating and recording the infrared transmittance of the components of a mixture, the apparatus and method may also be used, in combination with other suitable instrumentation, to control a variety of reactions in the field of petroleum refining, chemical manufacture, etc.

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The authors wish to thank the Leeds & Northrup Company for making a special recorder to meet their requirements, and to acknowledge the help of Ben Pitman, formerly of the Perkin-Elmer Corporation, in designing the turret mechanism.

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Determination of 1,2 Addition in Polymers and Copolymers of Butadiene

Infrared Spectrometric Method

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THE structural features of synthetic rubberlike polymers evidently have pronounced effects on many of the physical properties exhibited by these materials. Unfortunately, not enough is known about physical behavior in relation to structure and it is of some importance that the subject be investigated. Before relationships between structure and properties can be established, however, it is necessary to develop satisfactory methods for the detection and measurement of possible structural differences.

For polymers and copolymers of 1,3-butadiene, the manner in which the butadiene units are linked within the molecular chain constitutes one of the important structural features. Thus, a butadiene unit might be present in a so-called 1,4 configuration or as a 1,2 unit, which results in a vinyl side chain. The presence of units of the latter type may be responsible for some of the undesirable characteristics of synthetic rubbers.

During recent years the problem of estimating the degree to which 1,2 addition has occurred in the polymerization of butadiene polymers has been attacked by several different chemical and physical methods with varying degrees of success. A method involving perbenzoic acid addition and the infrared spectrometric approach appears to be the most promising. The present study was undertaken with the object of developing a satisfactory infrared spectrometric method for the quantitative determination of the fraction of butadiene units which have undergone 1,2 addition in polybutadiene and butadiene-styrene copolymers.

Although the complete infrared spectra of any two nonenantiomorphic compounds must be different, certain absorption bands are often characteristic of particular structural groups and are not markedly affected in position and intensity by the environment of the group. This fact provides the basis for all infrared spectrometric methods for the determination of the relative or absolute degree of 1,2 addition in polymers.

Swaney and White (*13*) chose the 970 and 990 cm.$^{-1}$ bands appearing in the spectra of polymers and copolymers of butadiene as indicative, respectively, of internal double bonds (—CH=CH—) or 1,4-butadiene units, and of vinyl groups or 1,2-butadiene units. They defined a "1,2 index" as a function of the optical densities at 970 and 990 cm.$^{-1}$ measured for polymer films with appropriate corrections for overlap.

Field, Woodford, and Gehman (*5*) approached the problem of determining the percentage of 1,2 addition of butadiene polymers and copolymers through the use of an "intensity ratio," a function of the relative intensities of the 996 and 967 cm.$^{-1}$ bands meas-

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A spectrometric method for the measurement of 1,2 addition in butadiene-containing polymers is described. The procedure for polybutadienes depends upon the measurement of infrared absorption intensities of the 910 cm.$^{-1}$ band, considered characteristic of vinyl groups. The ratio of the concentration of vinyl groups to that of butadiene units in the solution examined is taken to be the fraction of such units having undergone 1,2 addition. The extinction coefficient at the wave number 910 cm.$^{-1}$ of the 1,2 units of polybutadienes is assumed to be equal to that determined for three olefins containing vinyl groups. The extinction coefficient of the 1,4 units at this wave number is considered negligible, thus permitting the computation of vinyl-group molarities in polybutadiene solutions from optical density measurements. The extension of the method to butadiene-styrene copolymers requires compositional analyses, which can be accomplished spectrometrically through the use of the 700 cm.$^{-1}$ band, characteristic of the phenyl group. A correction is made for the contribution of the polybutadiene portion to the absorption at this wave number. The vinyl-group concentrations of the copolymer solutions are computed by the authors, as for the polybutadiene solutions, through the measurement of the optical densities at 910 cm.$^{-1}$

ured for polymer films. This ratio was calibrated by use of mixtures of 1- and 2-octene as standards, using the same band assignments employed by Swaney and White.

Rasmussen and Brattain (*11*) propsed a method for the determination of per cent 1,2 values for polymers and styrene copolymers of butadiene which depended on the optical densities at 912, 967, and 994 cm.$^{-1}$ of chloroform solutions of the polymers. The intensity of the 967 cm.$^{-1}$ band was considered to be indicative of the amount of 1,4 units; the intensities of the 912 and 994 cm.$^{-1}$ bands were used to indicate the relative amounts of 1,2 units. These bands were calibrated through measurements made on 1-hexene, 1-heptene, and 3-octene.

The infrared methods thus far developed for determining the degree of 1,2 addition in polymers and copolymers of butadiene have all been based in part upon the absorption band appearing at about 967 cm.$^{-1}$, attributed to the internal double bonds of the 1,4 units. Except for recent work by Hart and Meyer (*6*), no mention has been made of the relative contributions of the cis and trans configurations. Apparently it has been tacitly assumed either that the two forms have the same extinction coefficient at this wave number, or that the cis-trans ratios of butadiene polymers and copolymers all have the same value. Those methods which are considered to yield absolute per cent 1,2 values require the additional assumption that the invariant cis-trans ratio of the polymers is equal to that of the internal olefins employed as standards.

Rabjohn, Bryan, Inskeep, Johnston, and Lawson (*10*) proposed a chemical method for measurement of 1,2 addition based on the ozonolysis of butadiene polymers. However, as the reactions were not quantitatively clear cut, the authors designated their apparent per cent 1,2 values as "ozonization numbers." Possibly the best method so far proposed for the measurement of percentage of 1,2 addition is the perbenzoic acid titration method originally worked out in Germany (*17*) and further developed by Kolthoff and Lee (*7*) and Saffer and Johnson (*12*).

The present investigation was undertaken with the intention of developing a spectrophotometric method free from unwarranted assumptions regarding cis-trans ratios.

The experimental work was carried out using an automatic-recording Perkin-Elmer infrared spectrometer, Model 12 B, employing sodium chloride prism and windows. The spectra of liquids were determined while they were contained in sodium chloride cells of either 0.025- or 0.050-mm. inside thickness; polymeric samples were examined as films of about 0.03-mm. thickness spread on a single salt plate.

The determination of per cent 1,2 addition in polymers did not require measurement of complete spectra but only measurement of intensities at particular wave lengths. For this purpose carbon disulfide solutions were used. Carbon disulfide was chosen because it is fairly transparent, even for the 1.7-mm. cell employed, in the useful 900 to 1000 cm.$^{-1}$ and 675 to 725 cm.$^{-1}$ regions.

The spectra of ten cis and trans olefins of the type RCH=CHR′ were measured from 650 to 3500 cm.$^{-1}$: *cis*-2-hexene, *trans*-2-hexene; *trans*-3-hexene; *cis*-2-octene, *trans*-2-octene; *cis*-3-octene; *cis*-4-octene, *trans*-4-octene; *cis*-5-decene, *trans*-5-decene (these were kindly supplied by K. N. Campbell, University of Notre Dame, who with L. T. Eby had prepared them from the corresponding alkynes, *4*). The cis forms were said to be about 95 mole % pure; the purity of the trans forms was about 99 mole %. The impurities in the former are largely the corresponding trans isomer (*3*).

Four spectral regions were observed in which the cis forms differed markedly from the trans: in the neighborhoods of 700 to 740 cm.$^{-1}$, 967 cm.$^{-1}$, 1405 cm.$^{-1}$, and 1650 to 1670 cm.$^{-1}$ (*2*). Of these only the 967 cm.$^{-1}$ region is discussed here, because that has been used for the determination of 1,2 addition.

Each of the olefins examined appears to absorb radiation at about 967 cm.$^{-1}$ The band is very strong for each trans olefin but of only moderate strength for the cis forms. The intensity of the 967 cm.$^{-1}$ band for the trans forms is so great that the presence of small amounts of trans impurities in the cis olefins would make this band appear. Hence it is difficult to conclude whether or not the 967 cm.$^{-1}$ band shown by the cis samples is strong enough to be indicative of absorption by the cis structure, but in any case the absorption at 967 cm.$^{-1}$ is very much more intense for the trans forms.

Although the number of measured spectra of cis and trans RCH=CHR′ olefins is still small, it is probable that the 967 cm.$^{-1}$ band exhibited by cis-trans mixtures of this type is almost entirely due to the trans form. It appears, therefore, that the assumptions with respect to the 967 cm.$^{-1}$ band, upon which most of the previous infrared spectrometric investigations have been based, are probably unsound. Inasmuch as cis and trans

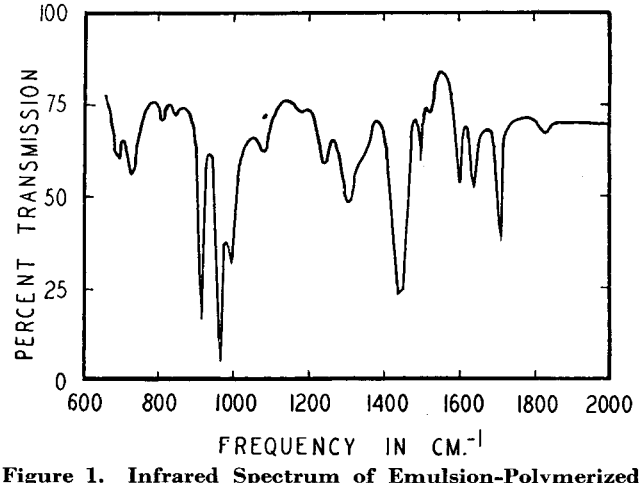

Figure 1. Infrared Spectrum of Emulsion-Polymerized Polybutadiene

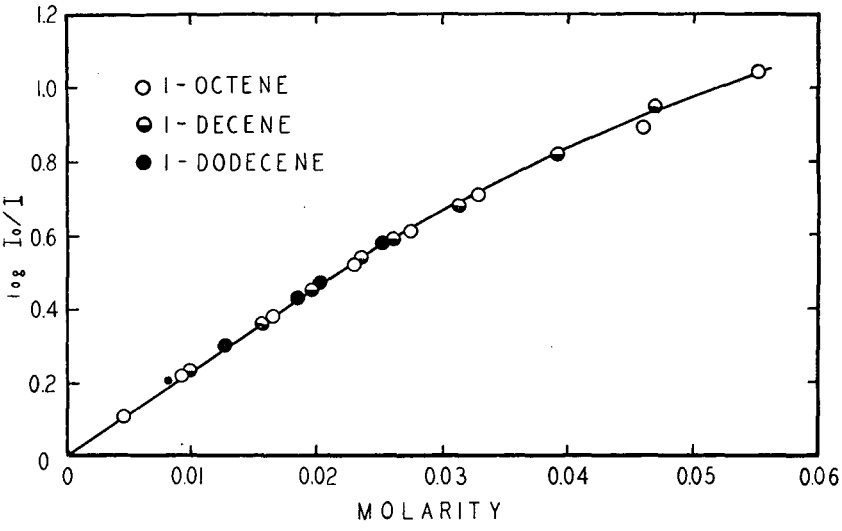

Figure 2. Optical Densities of Three Vinyl Group Olefins at 910 Cm.$^{-1}$

olefins of sufficient purity for accurate measurement of their 967 cm.$^{-1}$ extinction coefficients are not at present available, it is desirable to develop an infrared method for determining the percentage of 1,2 addition which does not depend upon measurements at 967 cm.$^{-1}$

PROPOSED METHOD FOR DETERMINATION OF PERCENTAGE OF 1,2 ADDITION IN POLYBUTADIENES

Direct measurement of 1,2 units should be feasible if the spectra of polybutadienes exhibit a strong band characteristic of vinyl groups which receives little or no contribution from other structural features. Absorption bands at about 995 and 1830 cm.$^{-1}$, attributed to vinyl groups, appear in the polybutadiene spectra (Figure 1). The former, however, appears to be overlapped by the 967 cm.$^{-1}$ band, and the latter is of very low intensity.

Of the other bands in the spectra of polybutadienes, the only strong band that has not been definitely assigned to structures other than vinyl groups is that at about 910 cm.$^{-1}$ This has been variously attributed to any carbon-carbon double bond (*16*, *18*) or to vinyl groups (*11*, *14*, *15*). Examination of the published spectra (*1*) of 14 noncyclic olefins, as well as spectra of five others (1-decene, 1-dodecene, 1-tetradecene, 1-hexadecene, and 1-octadecene) measured in the course of the present work, indicates that, with the exception of ethylene, vinyl group olefins exhibit a very strong band between 909 and 916 cm.$^{-1}$, usually in 909 to 912 cm.$^{-1}$ region. The spectra (*1*) of ten noncyclic olefins of the RCH=CHR′ type show relatively little tendency toward preferential absorption in the immediate neighborhood of 910 cm.$^{-1}$ All but a few of 78 noncyclic paraffins (*1*) show very little absorption at 910 cm.$^{-1}$ compared to the characteristic methylene absorption at about 1470 cm.$^{-1}$, although the absorption at 910 cm.$^{-1}$ of 1-alkenes, even as large as 1-octadecene, and of polybutadienes is greater than that at about 1470 cm.$^{-1}$ It is thus considered probable that the 910 cm.$^{-1}$ band exhibited by polybutadienes can be considered characteristic of the vinyl groups and to receive no significant contributions from other structural features.

The optical densities of carbon disulfide solutions of 1-octene, 1-decene, and 1-dodecene were measured at molarities up to about 0.055 in a cell of 1.7-mm. inside thickness. The optical density (log I_0/I) versus molarity curves (Figure 2) based on these results are superposable within experimental error and are linear at molarities below about 0.025 with a slope of 22.9 liters per mole. The superposability of the curves indicates that alteration in the concentration of methylene groups does not appreciably affect the absorption at 910 cm.$^{-1}$ and suggests that the extinction coefficient at 910 cm.$^{-1}$ of 1-alkenes can be assigned to the vinyl group in any nonconjugated hydrocarbon environment.

On the basis of the facts and considerations cited above, the following method is proposed for the determination of the percentage of 1,2 units in any polybutadiene:

The optical density at 910 cm.$^{-1}$ of the polymer in carbon disulfide solution is measured in the concentration range for which the optical density values fall between about 0.3 and 0.6, which is the range for maximum experimental accuracy. The optical density values are plotted against the corresponding butadiene-unit molarities, which are determined by evaporation of aliquot portions of the solution. The ratio of the slope of this curve to that of the 1-alkenes used as standards is taken as the fraction of 1,2 addition for the sample of polybutadiene.

The validity and accuracy of this method depend upon the following assumptions and approximations:

1. The fraction of butadiene units which have added in a 1,2 manner is equal to the corresponding fraction for the soluble portions of the polymer. (This assumption is necessary only for samples containing gel.)
2. The number of vinyl groups in the soluble portion is equal to the number of butadiene units in that portion which have added in the 1,2 manner.
3. For a given cell thickness, the intensity of absorption at 910 cm.$^{-1}$ of the polybutadiene solutions depends only on the concentration of vinyl groups.
4. The molar extinction coefficient at 910 cm.$^{-1}$ of 1-octene, 1-decene, and 1-dodecene is equal to that of the vinyl groups of polybutadienes.

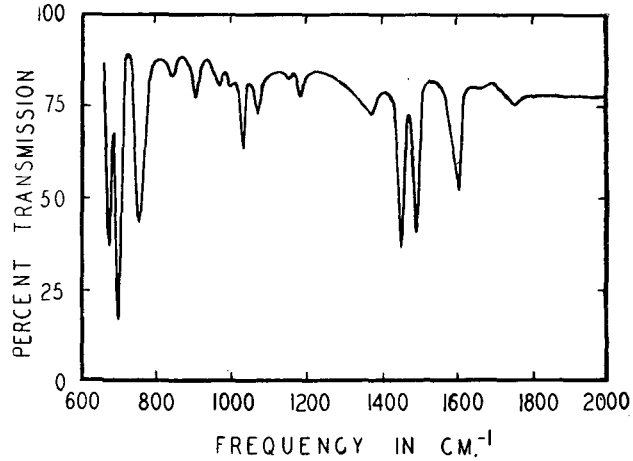

Figure 3. Infrared Spectrum of Polystyrene

The first two assumptions are not considered subject to appreciable error, but the latter two may be more serious. They cannot be directly tested without standard polybutadiene samples of accurately known compositions with respect to 1,2 and 1,4 units. The facts upon which the third assumption is based have been previously discussed. The fourth is based mainly upon the observed equality of the extinction coefficients of 1-octene, 1-decene, and 1-dodecene.

EXTENSION OF METHOD TO BUTADIENE-STYRENE COPOLYMERS

The infrared spectra of copolymers generally have the appearance of appropriately weighted averages of the sepctra of the polymers of the individual monomers (Figures 1, 3, and 4). This

is not to be interpreted as suggesting that the copolymers are mechanical mixtures of the separate polymers, but rather is indicative of the insensitivity of infrared absorption to chain structure. It is nevertheless convenient to speak of the "polystyrene portion" or the "butadiene portion" of the copolymers, without intending to imply that these portions are molecularly distinct.

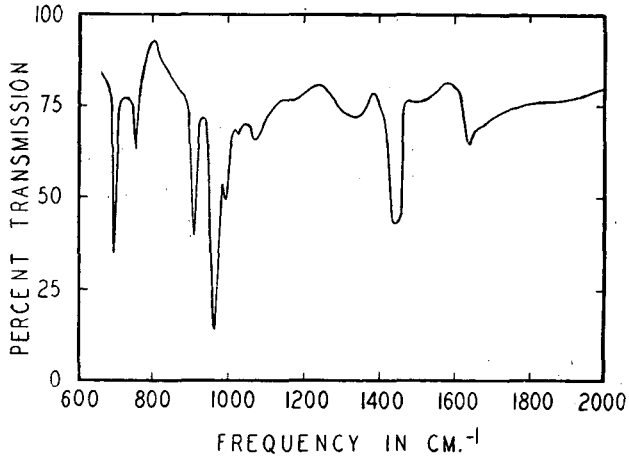

Figure 4. Infrared Spectrum of Emulsion-Polymerized Butadiene-Styrene Copolymer (GR-S)

The extension of the method to the copolymers of butadiene requires in addition to the usual measurements the determination of the weight per cent of the polybutadiene portion and a correction for the absorption at 910 cm.$^{-1}$ due to the other portion. The calibration of a strong band characteristic of the polycomonomer would render feasible the determination of the percentages of the two portions, assuming any necessary correction can be made for the contribution of the polybutadiene to this particular band. The optical density at 910 cm.$^{-1}$ could then be corrected for the absorption due to the nonpolybutadiene portion, if this were appreciable, and the percentage of 1,2 butadiene units computed.

The spectra of polystyrene (Figure 3) and of butadiene-styrene copolymers (Figure 4) show a strong band at about 700 cm.$^{-1}$ This band is considered by White and Flory (*18*) to be of aromatic origin. Examination of a great many spectra (*1*) makes it appear likely that the 700 cm.$^{-1}$ band is characteristic of the phenyl group and receives little or no contribution from saturated hydrocarbon structures.

The optical densities at 700 (Figure 5) and 910 cm.$^{-1}$ of two polystyrene samples were measured in carbon disulfide solutions of various concentrations. The two samples gave similar results at each of these wave numbers. The slopes of the curves obtained by plotting optical density at 910 cm.$^{-1}$ against concentration are small and approximately equal (about 0.01 liter per gram). Thus, the correction required at 910 cm.$^{-1}$ for the contribution of the polystyrene portion to the absorption of 75/25 butadiene-styrene copolymers is almost negligible.

The optical densities at 700 cm.$^{-1}$ of the carbon disulfide solutions of six emulsion- and three sodium-polymerized polybutadienes were measured at various butadiene-unit molarities. The slopes of the curves of optical density versus concentration are small and approximately equal (about 0.015 liter per gram); thus, the correction required at 700 cm.$^{-1}$ for the contribution of the polybutadiene portion to the absorption of butadiene-styrene copolymers is also small.

The determination of the percentage of 1,2 units in the butadiene portion of the butadiene-styrene copolymers involves the same experimental procedure used for the polybutadienes except that measurements of optical densities at 700 cm.$^{-1}$ are also made. The evaluation of the weight per cent of the polystyrene fraction is an intermediate step in computing the 1,2 values.

The measured optical density at 700 cm.$^{-1}$ allows the computation of a first approximation for the weight per cent of the polystyrene portion and hence of the polybutadiene portion. From this first approximation to the weight per cent of polybutadiene a correction is made for the polybutadiene contribution to the absorption of the styrene portion at 700 cm.$^{-1}$ From this corrected optical density at 700 cm.$^{-1}$ a second approximation is obtained for the weight per cent of the polystyrene. Further successive approximations can be made until a satisfactory coincidence is obtained. The optical density at 910 cm.$^{-1}$ is then corrected for the contribution of the polystyrene fraction and the percentage of 1,2 units in the polybutadiene portion is computed.

ANALYTICAL RESULTS

The above methods of analysis were applied to several emulsion- and sodium-polymerized polymers and styrene copolymers of butadiene. A few of these polymers had been analyzed for per cent 1,2 units by Saffer and Johnson (*12*) or Meehan (*9*) by the perbenzoic acid titration method. Results appear in Table I.

EVALUATION OF METHOD OF ANALYSIS

If measurements are made on several solutions of different concentrations in the optimum range, the 1,2 values calculated for the polybutadienes can be reproduced within one percentage point. The method is subject to several possible systematic errors. If the vinyl-group olefins used as standard were only 95 weight % 1-alkene (the minimum purity claimed by the supplier) and if the impurities were essentially transparent at 910 cm.$^{-1}$, the 1,2 values measured would be about 1 and 3 percentage points too high for the emulsion- and the sodium-polymerized polymers, respectively. If nonvinyl components of the polybutadienes contribute appreciably to the absorption at 910 cm.$^{-1}$, the 1,2 values measured will tend to be too high, the higher 1,2 values being subject to a smaller fractional error than the lower ones. Any attempt to evaluate and correct for possible absorption due to the internal double bonds of the 1,4 units by measurements on commercially available 2-alkenes would be likely to result in a large overcorrection due to the presence of strongly absorbing 1-alkene impurities usually present in such samples. Because it is not possible to estimate *a priori* the maximum error due to absorption of the nonvinyl-group portion of the polymers, it must be recognized that the positive error may be considerable. Systematic negative error, however, is improbable.

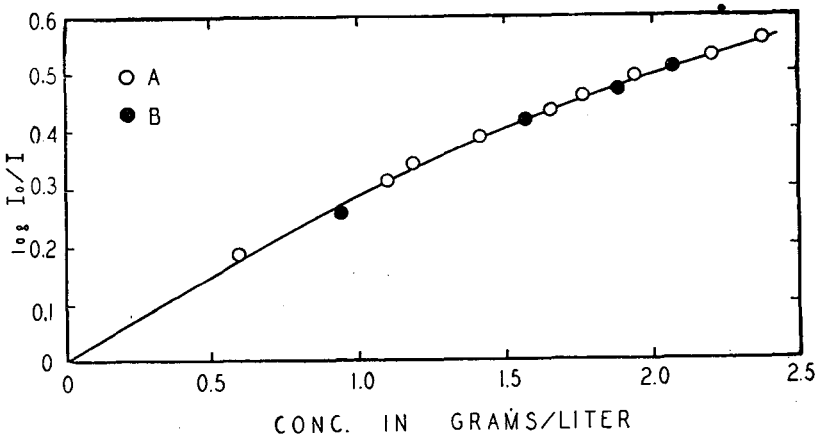

Figure 5. Optical Densities of Two Samples of Polystyrene at 700 Cm.$^{-1}$

Table I. Analyses for 1,2 Units of Polymers and Styrene Copolymers of Butadiene

Polymer Type	Sample No.	Weight % Polystyrene Portion by Infrared Method	% 1,2 by Infrared Method	% 1,2 by Perbenzoic Acid Titration Method
Polybutadiene				
Emulsion-polymerized	1	..	19	...
	2	..	19	...
	3	..	20	...
	4	..	21	25[a]
	5	..	22	...
	6	..	24	25.2[b]
Sodium-polymerized	7	..	70	...
	8	..	71	59.1[b]
	9	..	71	...
Butadiene-styrene copolymers				
Emulsion-polymerized	10	20	20[c]	22[a]
	11	20	20[c]	...
	12	20	22[c]	23.3[b]
Sodium-polymerized	13	26	65[c]	...
	14	24	72[c]	...

[a] Results of Saffer and Johnson (*12*).
[b] Results of Meehan (*9*).
[c] Percentages refer to butadiene portion of copolymer.

For emulsion polybutadienes, 1,2 values of about 20% have been obtained by the perbenzoic acid titration method (*8*), by the intensity ratio infrared method (*5*), and by the present method. The values obtained for sodium-polymerized polybutadienes by the various methods show much less agreement. Values ranging from 58 to 74% have been obtained by the perbenzoic acid method (*17*), about 80% by certain infrared methods (*5*, *11*), and about 70% by the present method. However, except for a few examples cited in Table I, the above comparisons were not for the same polymer samples.

The analysis of the butadiene-styrene copolymers is much less direct than that of the polybutadienes and hence might be expected to be less satisfactory. Nevertheless, if the intermediate determination of the per cent polystyrene were in error by as much as 20% the resulting error in the 1,2 values would be off by only 1 and 3 percentage points, respectively, for the emulsion- and the sodium-polymerized copolymers. The only other additional source of error for copolymers not involved for polybutadienes is the correction applied to the optical densities at 910 cm.$^{-1}$ of the copolymer solutions for the absorption due to the polystyrene portion. This correction is very small; either omitting or doubling it would alter the 1,2 values by about 0.5 percentage point. It is believed, therefore, that the 1,2 values measured for the styrene copolymers are of the same order of accuracy as those determined for the polybutadienes.

ACKNOWLEDGMENT

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Determination of Dimethyl Ether in Methyl Chloride

A Combination Infrared and Chemical Method

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IN CERTAIN refinery operations it was necessary to detect very small concentrations of dimethyl ether in a stream containing methyl chloride as the major component and isobutylene (2-methylpropene) and isobutane (2-methylpropane) as the minor components. First consideration was given to an analytical distillation; this method, however, was unsatisfactory because of the proximity of the boiling points of dimethyl ether and methyl chloride (−23.65° and −24.22° C., respectively, *7*). Analytical distillation was further complicated by the formation of an azeotrope (*5*) consisting of isobutane and methyl chloride. Attempts at a chemical method (*8*) were also unsatisfactory because of both a lack of sensitivity and the presence of the above-mentioned interfering materials.

Attention was then turned to an investigation of the possibilities of analysis by infrared spectroscopy because of the dissimilarity between the ether molecule and the other components of the stream (*2*, *6*). Good sensitivity was considered likely because of the usually strong absorption in the 8- to 10-micron region of oxygenated compounds as compared to hydrocarbons or chlorinated hydrocarbons (*1*). A literature survey indicated the dimethyl ether (*6*) to have strong absorption in the regions around 1200 and 900 cm.$^{-1}$ In both these ranges the absorption due to methyl chloride (*2*) was of little or no significance. Further investigation of the spectra of methyl ether and methyl chloride on the Beckman routine infrared spectrophotometer (*3*) in the regions just stated confirmed the literature observations that

Both an infrared absorption procedure and a combination of the infrared method with a chemical concentration procedure are given for the determination of small quantities of dimethyl ether in admixture with methyl chloride and 2-methylpropene. The precision of the infrared method alone is ±0.004%, while the combination of the two procedures gives a precision of ±3 to 5 p.p.m., depending on the degree of concentration.

methyl chloride was practically transparent in both regions, whereas isobutylene absorbed rather strongly in the 900 cm.$^{-1}$ region but weakly in the 1200 cm.$^{-1}$ region. Dimethyl ether was found to have two peaks at 1200 and 1180 cm.$^{-1}$, respectively, where the extinction coefficient was exceptionally high (Figure 1). These were selected as the most likely frequencies for this determination. The approximate extinction coefficients at each of these frequencies for all the components likely to be present were measured using various pressures of the pure material and are listed in Table I. The cell length was omitted in the calculation, as the same instrument and cell was to be used for all analyses.

Table I. Extinction Coefficients

Component	1200 Cm.$^{-1}K^a$	1180 Cm.$^{-1}K^a$
Dimethyl ether	40	40
Methyl chloride	0.0	0.0
Isobutylene	0.1	0.2
Isobutane	0.4	3.0

[a] $K = \frac{\text{optical density} \times 1000}{\text{pressure in mm. Hg}}$

EXPERIMENTAL

Using the approximate extinction coefficient for dimethyl ether given in Table I and assuming the validity of Beer's law with the smallest detectable optical density as 0.005, the minimum detectable amount of ether can be calculated to be about 0.015 mole %. However, it was impossible to obtain accurate determinations of the extinction coefficient using the pure ether because of its excessive absorption. Accurate preparation of synthetic mixtures for calibration was therefore necessary to obtain a partial pressure of ether low enough to be comparable to that determined in the sample. [Good accuracy in the preparation of the synthetic samples is obtained by weighing a small amount of ether in a sealed U-shaped, heavy-walled, capillary tube. The tube is then broken below the surface of a large quantity of pure liquid methyl chloride (about 500 ml.). The material is stirred and pulled into a chilled evacuated bomb.] Straight-line calibration curves prepared from measurements made on these synthetics can then be used directly without the calculation of extinction coefficients. Inspection of such curves shows the minimum detectable amount to be about 0.007 weight % dimethyl ether (again assuming 0.005 to be the minimum detectable optical density).

In Table II is listed a series of synthetic samples containing about 10% isobutylene as an interfering material, which were analyzed by this method. The greatest deviation from the known ether content for this series of synthetic mixtures is 0.004%, indicating excellent accuracy for the determination. However, under the most ideal conditions the optical density measurements have a maximum reproducibility of about 0.004 for this type of instrument. This optical density is equivalent to 0.007 weight % ether. For this reason the maximum reproducibility claimed for this analysis is ±0.007%.

In order to improve the accuracy and sensitivity of the method for low concentrations of the ether, a concentration procedure, utilizing the fact that dimethyl ether forms a stable complex with boron trifluoride (*4*), was developed for use before analysis by infrared absorption. In this case the sample is treated with boron trifluoride to form the ether complex, which remains as a solid salt after the evaporation of the unreacted portion of the sample. The salt is then decomposed with aqueous sodium hydroxide and recovered in pure methyl chloride in a concentration 20 to 25 times greater than the original. Isobutylene interferes with this procedure, in so far as it forms a rubbery polymer in the presence of boron trifluoride from which it is difficult to strip the ether. The formation of this isobutylene polymer may be inhibited by the addition of a small amount of *o*-cresol.

The equations for these reactions may be written as follows:

$$BF_3 + (CH_3)_2O \longrightarrow BF_3 \cdot (CH_3)_2O$$

$$BF_3 \cdot (CH_3)_2O + 6NaOH \longrightarrow Na_3BO_3 + 3NaF + 3H_2O + (CH_3)_2O$$

The concentrated sample is then analyzed by infrared absorption and the ether calculated on the original basis. The method is good to about 3 to 5 p.p.m. on the original, assuming a concentration factor of 25 and complete recovery of the ether from the complex. Complete recovery, however, is not always realized; 90% is considered a good average recovery. For small concentrations of ether this 10% loss is not significant. Table III lists a series of synthetic samples concentrated in the above manner with the analysis of the concentrate by infrared absorption.

CALIBRATION OF INFRARED SPECTROPHOTOMETER

The dimethyl ether absorption peak is located by roughly scanning the spectra of ether from 8 to 10 microns. Careful scanning of the region of 1200 cm.$^{-1}$ (8.33 microns) reveals that dimethyl ether has two peaks, at 1200 and 1180 cm.$^{-1}$, respectively, which are suitable for analysis (Figure 1).

A series of known samples of dimethyl ether in pure methyl chloride ranging from 0.01 to 0.10 weight % is prepared in steps of 0.01%. Successive measurements of the optical density of each on the infrared spectrophotometer (*3*) at the two frequencies

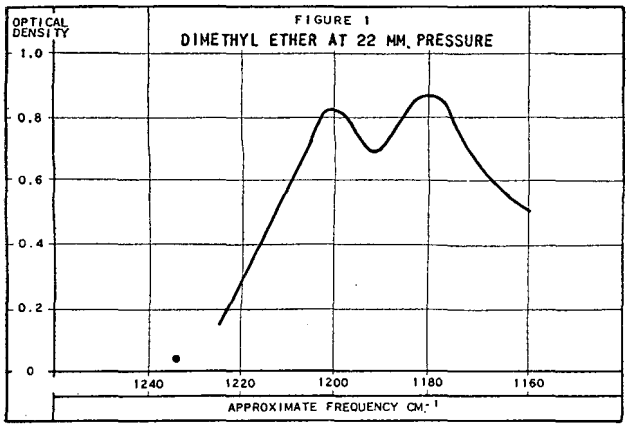
Table II. Synthetic Samples of Dimethyl Ether in Methyl Chloride and Isobutylene

Wt. % Iso-C_4H_8 by Synthesis	Wt. % Me_2O by Synthesis	Wt. % Me_2O by Analysis
10.0	0.036	0.034
10.0	0.054	0.055
15.0	0.017	0.015
10.0	0.036	0.032
10.0	0.054	0.057
0	0.020	0.024
0	0.004	0.003
0	0.007	0.009
0	0.011	0.012
0	0.004	0.008
0	0.007	0.006
0	0.011	0.009

Table III. Synthetic Samples

Me_2O by Synthesis, P.P.M.	Concentration Ratio (Approx.)	Me_2O Found in Concentrated Sample, P.P.M.	Me_2O Found in Sample on Original Basis, P.P.M.
0	25/1	0	0
23	21/1	420	23
46	20/1	840	42
92	9/1	740	85
31	25/1	700	28
31	20/1	480	24
46[a]	25/1	1250	50

[a] Contained 20 mole % isobutylene.



tane is made in the same manner. From these curves the coefficients given in Table IV are obtained.

PROCEDURE

Direct Determination. For analysis, measurements of the optical density at atmospheric pressure for the two frequencies 1200 and 1180 cm.$^{-1}$, are made and the proper correction (obtained from the coefficients given in Table IV) is applied for isobutylene (which may be determined by infrared absorption at 875 cm.$^{-1}$). The residual optical densities are then used to obtain the apparent ether content by reference to the previously prepared calibration curves. If the two figures obtained do not agree within the limits of reproducibility of the analysis, the sample may be assumed to contain some other interfering material for which a correction is necessary. Isobutane, which was found as an interfering material in many of the samples analyzed in this laboratory, was determined and corrected for in the following manner.

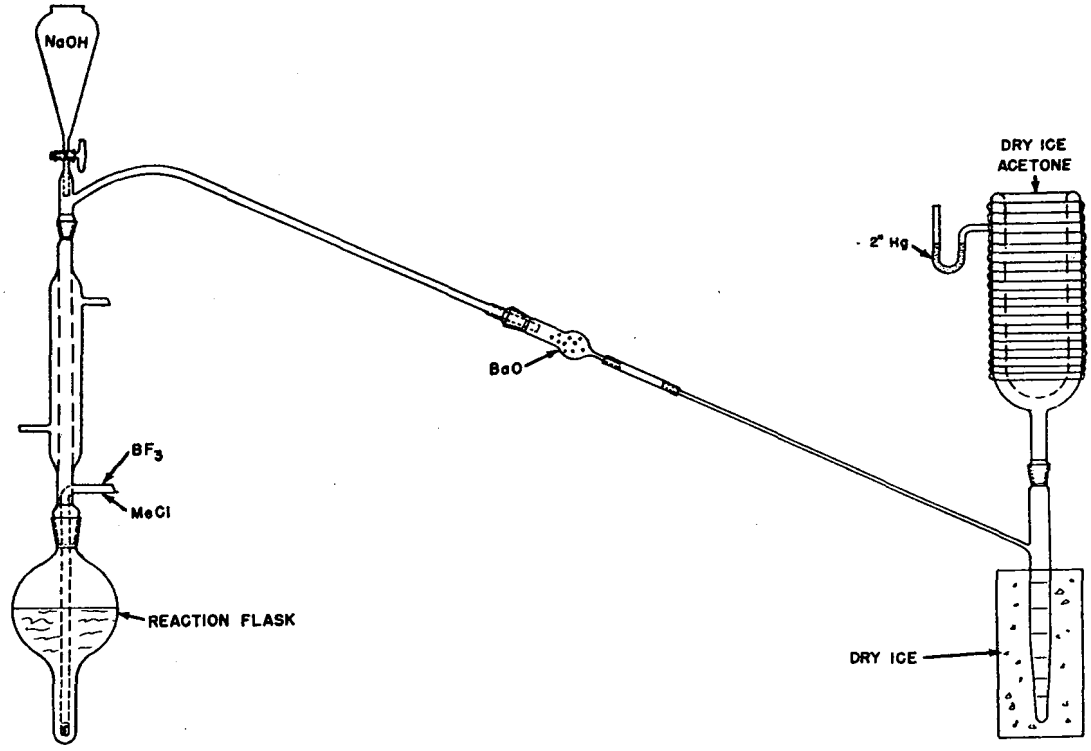


Figure 2. Apparatus for Concentration of Dimethyl Ether

indicated result in two straight-line calibration curves. (The reproducibility obtained on the optical density measurements of the same synthetic was ±0.004.)

To make correction for isobutylene the absorption of pure isobutylene at the two selected frequencies is measured at several different pressures, and the optical densities thus obtained are plotted against mole per cent isobutylene (calculated from the partial pressures) to obtain a straight line. Correction for isobutane is made in the same manner. From these curves the coefficients given in Table IV are obtained.

Table IV. Extinction Coefficients

	1180 Cm.$^{-1}K^a$	1200 Cm.$^{-1}K^a$
Isobutylene	10×10^{-2}	8.6×10^{-2}
Isobutane	2.65	0.57

$^a K = \frac{\text{optical density}}{\text{mol. fraction}}$.

The approximate per cent isobutane is calculated from its absorption coefficient at 1180 cm.$^{-1}$ given in Table IV by assuming all the absorption at 1180 cm.$^{-1}$ to be due to isobutane. This figure is used to obtain the correction for isobutane interference at 1200 cm.$^{-1}$ (Table IV). Subtraction of the absorption due to isobutane at 1200 cm.$^{-1}$ leaves only that due to dimethyl ether, the amount of which may then be obtained from the calibration curve at that frequency. The assumption that all the absorption at 1180 cm.$^{-1}$ is due to isobutane is justified only when the ratio of isobutane to ether is large, as was experienced in this work. If this ratio were decreased it would then become necessary to resort to the solution of simultaneous equations to obtain both the isobutane and ether figures.

Concentration. If the dimethyl ether is present in quantities below about 0.015%, it is convenient and more accurate to concentrate the ether by the following procedure:

The apparatus used for this procedure is shown in Figure 2. Moisture is eliminated (it will destroy the ether–boron fluoride complex) from the system by flushing with dry air. The reaction flask is charged with 500 ml. of cold sample measured to the nearest 20 ml. while taking the necessary precautions against the inclusion of moisture. The sample weight may be calculated from

the approximate density. If isobutylene is present it is advisable to add a few grams of *o*-cresol to inhibit the polymerization of isobutylene which makes the subsequent ether stripping operation difficult. The sample is then saturated with boron trifluoride until a white cloud of hydrolyzed boron trifluoride appears in the vent gases, indicating complete reaction of the ether. The unreacted methyl chloride is allowed to boil off by slowly warming the reaction flask while passing a small stream of pure methyl chloride through it. This vaporization should require about 20 minutes.

When vaporization is completed the system is flushed out with pure methyl chloride and the reflux head is cooled to about −70° F. with dry ice and acetone while flushing with methyl chloride is continued. When 10 to 12 ml. of methyl chloride have collected in the graduated receiver, 100 ml. of 10% sodium hydroxide are added dropwise to the reaction flask. The caustic solution is then stripped with a stream of methyl chloride until 20 to 25 ml. of methyl chloride have collected in the receiver. The concentrated sample is then transferred to a small evacuated bomb in which it may be held until ready for the infrared determination of the ether content.

ACCURACY AND PRECISION

By concentrating the sample in the manner described, the minimum detectable amount has been reduced to about 5 p.p.m. (depending on the degree of concentration), and the precision increased to the same figure (Table III). For the infrared method alone the minimum detectable is at best 0.007% with a maximum precision of ±0.004%. The precision here stated is true only when the concentration of the ether is in the range studied in this investigation, which was below 0.1%.

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Infrared Spectra of Sulfones and Related Compounds

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IN THE past decade the identification of structural features of organic molecules by means of infrared absorption spectra has been the subject of a large number of systematic investigations in which the absorption characteristics of many functional groups have been established. In the field of organic sulfur compounds, investigations of mercaptans (thiols), sulfides, and disulfides have established a sulfur-hydrogen stretching vibration (*5, 6, 18, 44*) in the region of 2500 cm.$^{-1}$, a carbon-sulfur stretching vibration (*43*) between 600 and 700 cm.$^{-1}$, and finally a sulfur-sulfur stretching vibration (*43*) in the neighborhood of 500 cm.$^{-1}$ Among inorganic sulfur compounds, sulfur dioxide (*31*) shows bands at 1151 and 1361 cm.$^{-1}$, respectively, which represent sulfur-oxygen stretching frequencies, while sulfur trioxide (*21*) shows three bands at 1065, 1205, and 1330 cm.$^{-1}$ Duval and Lecomte (*16, 17*) have reported that inorganic dithionates absorb at approximately 985 and 1200 cm.$^{-1}$

No systematic investigation of sulfur-oxygen absorption frequencies in organic molecules has been reported. Data on only a few isolated compounds have been published. Downing *et al.* (*15*) have investigated the spectra of bis-(*p*-chlorophenyl)-sulfone and of 2,2,2-trichloro-1-(*o*-chlorophenyl)-ethyl-*p*-chlorobenzenesulfonate in connection with the analytical determination of DDT in industrial manufacture. Barnes *et al.* (*3*) have recorded curves for orthanilic, metanilic, and sulfanilic acids, and *o*-, *m*-, and *p*-sulfanilamide. Adams and Tjepkema (*1*) have reported the spectra of sixteen *N,N'*-disubstituted-*N,N'*-dibenzenesulfonyldiaminomesitylenes and stated that they show an absorption between 1160 and 1180 cm.$^{-1}$ due to the —SO_2— group. Barnes *et al.* (*2*) in a table of functional group absorption frequencies have tentatively assigned the region of 1250 to 1350 cm.$^{-1}$ to sulfone absorption and the region of 1140 to 1200 cm.$^{-1}$ to the absorption of the sulfonate grouping. But no experimental data are presented in their paper.

This investigation was undertaken in order to obtain experimental data on the sulfur-oxygen stretching frequencies in sulfones and related compounds; no attempt was made to obtain a

Table I. Absorptions of Sulfones and Sulfides between 1100 and 1400 Cm.$^{-1}$

Diphenylsulfone		1110	1155	1166[a]		1310[a]	1325	
Diphenylsulfide	(*23*)			1166		1305		
Di-*n*-butylsulfone	(*25*)		1139	1175			1330	
Di-*n*-butylsulfide	(*8*)			1175	1220			
Methylethylsulfone	(*4*)		1145		1280		1330	1380
Methylethylsulfide	(*27, 28*)				1259			1383
Phenylbenzylsulfone	(*37*)	1128	1155		1258	1305[a]	1325	
Phenylbenzylsulfide	(*38*)				1242	1300		
Dibenzylsulfone	(*20*)	1120	1155	1205	1270		1325	
Dibenzylsulfide	(*39*)			1200	1245			
Di-(*n*-butylsulfonyl)-methane	(*40*)		1130				1340	
Di-(*n*-butylmercapto)-methane	(*40*)							
1,2,-Di-(phenylsulfonyl)-ethane	(*36*)	1141[a]	1159	1238				
1,2-Di-(phenylmercapto)-ethane	(*7*)			1221				
Diphenyldisulfone	(*24*)		1148	1170[a]	1202		1345	
Diphenyldisulfide	(*19*)				1201			
Phenylallylsulfone	(*35*)		1150	1184	1225	1302	1325	
Phenylallylsulfide	(*26*)			1175	1229			
Methylvinylsulfone	(*10*)		1140		1265[a]		1318	1387
Methylvinylsulfide	(*9*)				1258			1390
Bis-(*p*-hydroxyphenyl)sulfone	(*42*)	1102	1153	1180	1225	1286	1313	
Bis-(*p*-hydroxyphenyl)sulfide	(*41*)	1103		1171	1220	1280		
Thiodiglycolsulfone	(*29*)		1130	1170	1229	1285	1315	
Thiodiglycol				1157	1220	1285		
Thiodiglycol diacetatesulfone	(*33*)		1128		1240	1293	1325	
Thiodiglycol diacetate	(*33*)				1235	1295[a]		
p-Tolylallylsulfone	(*35*)		1150	1183		1301	1330	
Tri-(ethylsulfonyl)-methane	(*14*)	1134	1158	1175[a]	1231	1300[a]	1352	
Ethyl-β-(phenylsulfonyl)-acetate	(*32*)	1115	1153	1177	1275		1350	1380

[a] Well defined knee in curve at point indicated.

The infrared spectra in the region 1000 to 1500 cm.$^{-1}$ of thionyl chloride, sulfuryl chloride, sixteen sulfones, thirteen sulfides, two sulfoxides, two sulfates, two sulfonates, three sulfonic acids, three sulfonamides, and one sulfonyl chloride are reported. Characteristic bands for the —SO_2— group are discussed.

sulfur-oxygen bending frequency. Considering the data in the above-mentioned papers (with the exception of that by Adams, *1*, which was published while this work was in progress), as well as the Raman spectrum of sulfuryl chloride (*11, 30, 34*) which shows frequency shifts of 1182 and 1408 cm.$^{-1}$, it was decided to investigate carefully the region from 1000 to 1500 cm.$^{-1}$

EXPERIMENTAL

Instrument Used. The work described in this paper was performed using a Perkin-Elmer Model 12B infrared spectrometer with a breaker-type amplifier and a Brown strip-chart, electronic recorder. The instrument was used in the state of adjustment as supplied by the manufacturer. It was found that its resolving power was 20 to 25 cm.$^{-1}$ between 1000 and 1400 cm.$^{-1}$ under the conditions used, whereas a value of 8 to 11 cm.$^{-1}$ would have been expected from the data given in the manual supplied with the spectrometer. Approximately the latter resolving power is achieved with Halford's instrument in these laboratories, which, however, was not available for the work reported here. All spectra were taken using a sodium chloride prism. The sample cell employed consisted of rock-salt plates separated by a lead gasket of 0.101-mm. thickness (*12*).

All spectra were taken in solution. The solvents are indicated with each curve (Figures 1 to 6) and were commercial solvents that were dried and fractionated through a glass helix fractionating column prior to use. The concentrations ranged from 30 to 100 mg. of compound per cubic centimeter of solvent. The molar concentrations of corresponding sulfides and sulfones were the same, so that the extinctions are directly comparable. The final curves were obtained on a point by point basis from the ink tracings of the transmittances of the solution and of the solvent.

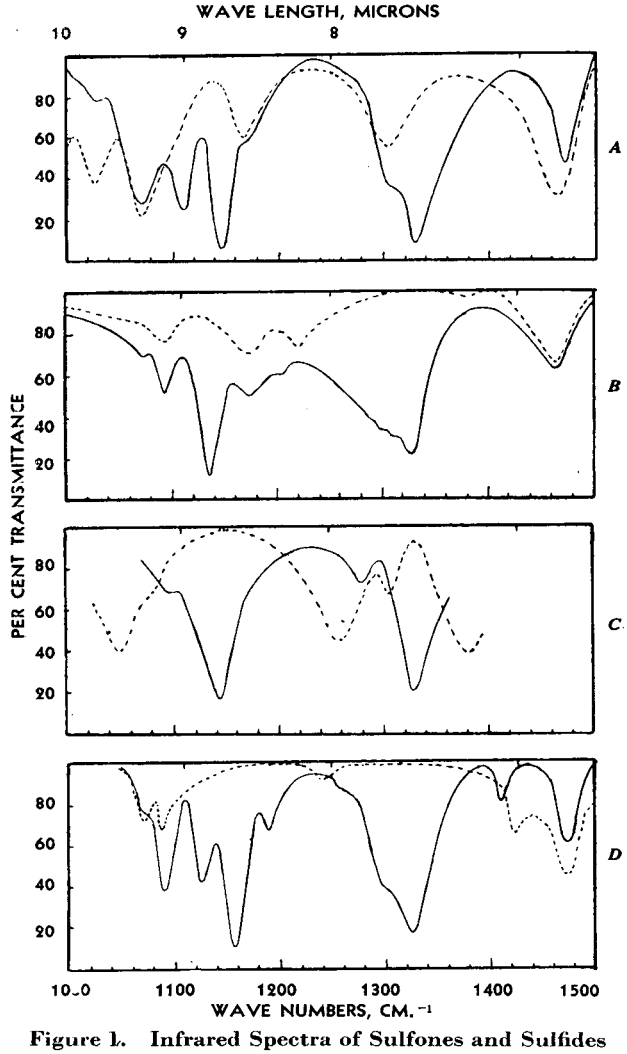

Figure 1. Infrared Spectra of Sulfones and Sulfides

A. —— Diphenylsulfone in chloroform and acetonitrile
- - - Diphenylsulfide in carbon tetrachloride
B. —— Di-*n*-butylsulfone in carbon tetrachloride
- - - Di-*n*-butylsulfide in carbon tetrachloride
C. —— Methylethylsulfone in acetonitrile
- - - Methylethylsulfide in carbon tetrachloride
D. —— Phenylbenzylsulfone in carbon tetrachloride
- - - Phenylbenzylsulfide in carbon tetrachloride

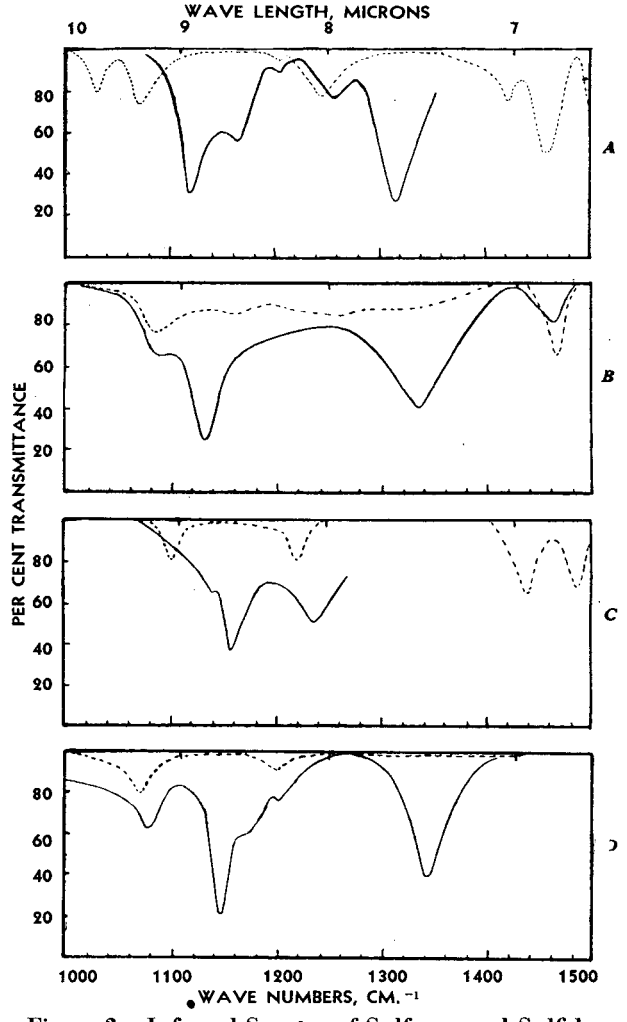

Figure 2. Infrared Spectra of Sulfones and Sulfides

A. —— Dibenzylsulfone in acetonitrile
- - - Dibenzylsulfide in carbon tetrachloride
B. —— Di-(*n*-butylsulfonyl)-methane in carbon tetrachloride
- - - Di-(*n*-butylmercapto)-methane in carbon tetrachloride
C. —— 1,2-Di-(phenylsulfonyl)-ethane in acetonitrile
- - - 1,2-Di-(phenylmercapto)-ethane in acetonitrile
D. —— Diphenyldisulfone in carbon tetrachloride
- - - Diphenyldisulfide in carbon tetrachloride

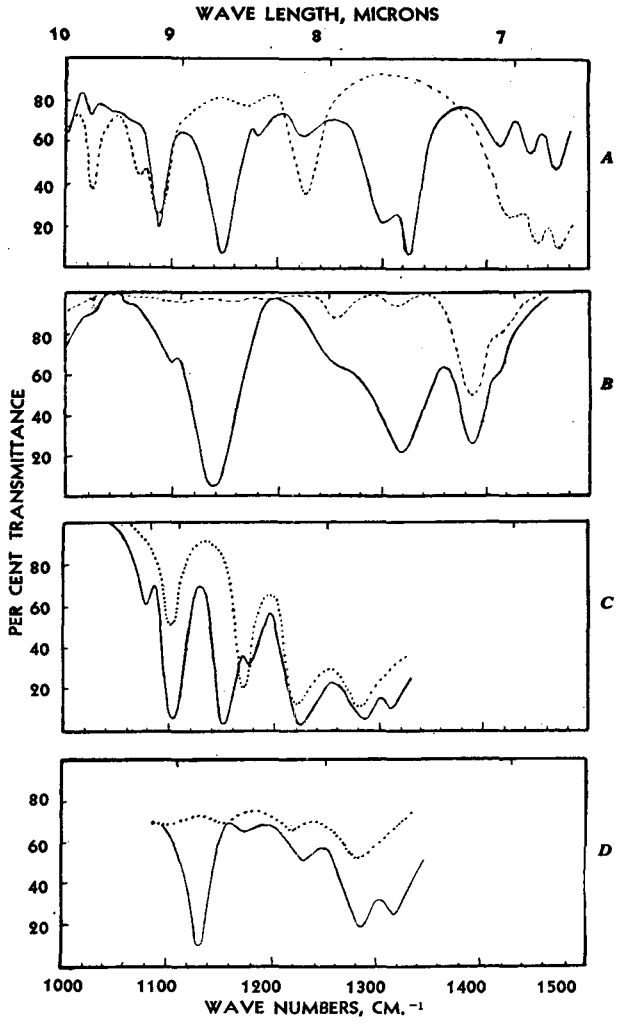

Figure 3. Infrared Spectra of Sulfones and Sulfides

A. —— Phenylallylsulfone in carbon tetrachloride
- - - Phenylallylsulfide in carbon tetrachloride
B. —— Methylvinylsulfone in carbon tetrachloride
- - - Methylvinylsulfide in carbon tetrachloride
C. —— Bis-(*p*-hydroxyphenyl)-sulfone in acetonitrile
- - - Bis-(*p*-hydroxyphenyl)-sulfide in acetonitrile
D. —— Thiodiglycolsulfone in acetonitrile
- - - Thiodiglycol in acetonitrile

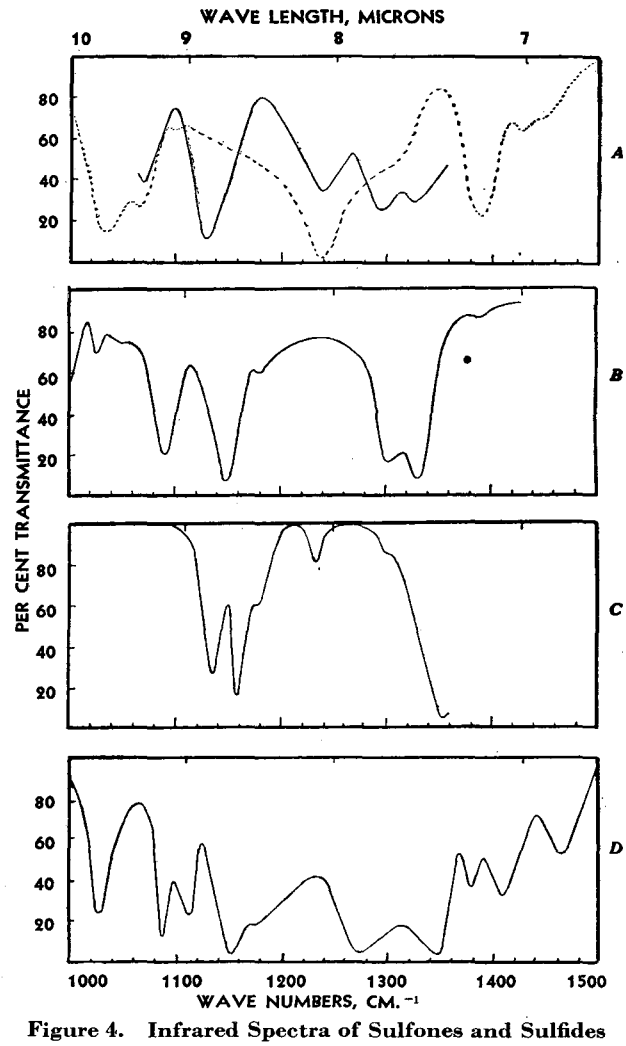

Figure 4. Infrared Spectra of Sulfones and Sulfides

A. —— Thiodiglycol diacetatesulfone in acetonitrile
- - - Thiodiglycol diacetate in carbon tetrachloride
B. *p*-Tolylallylsulfone in carbon tetrachloride
C. Tri-(ethylsulfonyl)-methane in acetonitrile
D. Ethyl-β-(phenylsulfonyl)-acetate in acetonitrile

The methods of preparation of the compounds are indicated by literature references in the tables. Diphenylsulfone, benzenesulfonic acid, *p*-toluenesulfonic acid, benzenesulfonyl chloride, the sulfonamides, and the sulfonates were obtained from the Eastman Kodak Company.

DISCUSSION OF RESULTS

The spectra of thirteen sulfones and of the corresponding sulfides have been examined for bands characteristic of the sulfone grouping. Each of the sulfones shows two which are absent in the corresponding sulfides. These absorptions occur between 1120 and 1160 cm.$^{-1}$ and between 1300 and 1350 cm.$^{-1}$ (see Table I). Both are strong absorptions, the one at the longer wave length (8.6 to 8.9 microns or 1120 to 1160 cm.$^{-1}$) usually being slightly stronger. No sulfides have been investigated which have absorptions in these regions due to absorptions of other parts of the molecule, such as naphthyl and metasubstituted phenyl sulfides, because the bands in the corresponding sulfones would be either unresolved or difficult to interpret.

Throughout this work the difficulty of finding a satisfactory solvent was encountered. Although carbon tetrachloride is excellent because it has only weak absorptions in the region investigated, many of the sulfones and of the compounds discussed below are not sufficiently soluble in this solvent. Chloroform can be used, but it has an absorption band at 1216 cm.$^{-1}$ which obliterates the region between the two frequencies in question. Acetonitrile, a solvent in which most of the investigated compounds are sufficiently soluble, unfortunately has two strong absorptions at 1040 and 1420 cm.$^{-1}$ The second absorption is so strong, that, with the cell used, any pattern of the solute above 1350 cm.$^{-1}$ is masked.

Table II. Absorption of Compounds Containing —SO_2— Group

Benzenesulfonamide	1100	1167		1301[a]	1358	
o-Toluenesulfonamide	1140	1162		1285	1346	
p-Toluenesulfonamide	1100	1167		1300	1358	
Methyl *p*-toluenesulfonate	1100	1185	1198	1315	1375	
n-Butyl *p*-toluenesulfonate	1100	1185	1198	1315	1370	
Benzenesulfonic acid	1100	1182	1261			
Ethanesulfonic acid		1171	1242	1300		
p-Toluenesulfonic acid	1100	1170	1250	1300		
Benzenesulfonyl chloride	1120	1185	1195	1322	1340	1390
Dimethylsulfate		1193				1412
Diethylsulfate	1161	1187				1415
Sulfuryl chloride	1128	1160	1196			1419

[a] Well defined knee in curve at point indicated.

It is therefore hard to interpret the spectrum of a compound in the region of the second absorption of the sulfone grouping.

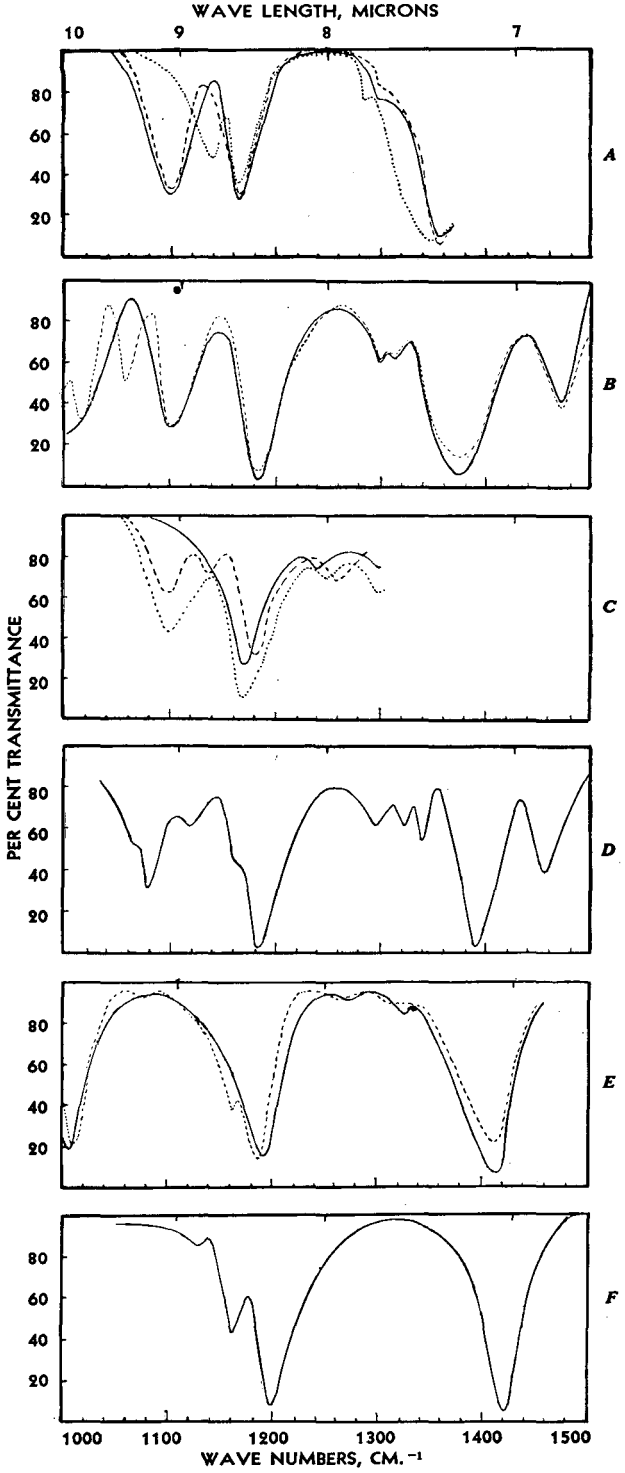

Figure 5. Infrared Spectra of Compounds Containing —SO_2— Group

A. - - - Benzenesulfonamide in acetonitrile
- - - *o*-Toluenesulfonamide in acetonitrile
—— *p*-Toluenesulfonamide in acetonitrile
B. —— Methyl *p*-toluenesulfonate in carbon tetrachloride
- - - *n*-Butyl *p*-toluenesulfonate in carbon tetrachloride
C. —— Ethanesulfonic acid in acetonitrile
- - - Benzenesulfonic acid in acetonitrile
- - - *p*-Toluenesulfonic acid in acetonitrile
D. Benzenesulfonyl chloride in carbon tetrachloride
E. —— Dimethylsulfate in carbon tetrachloride
- - - Diethylsulfate in carbon tetrachloride
F. Sulfuryl chloride in carbon tetrachloride

A number of other compounds, which are not sulfones but contain the —SO_2— grouping, have been investigated, and their absorption is shown in Table II.

The band at 1370 cm.$^{-1}$ in the spectra of the sulfonates is a broad band stretching over about 20 cm.$^{-1}$, and may be a doublet unresolved by the instrument. No second absorption was observed in the spectra of sulfonic acids up to 1335 cm.$^{-1}$, above which the absorption of the acetonitrile made it impossible to draw any conclusions. The peaks in sulfuryl chloride correspond to the Raman shifts (*11*, *31*, *43*) which are to be expected, as this molecule belongs to the point group C_{2v} in which these absorptions are both infrared and Raman active.

In Table III the absorptions of three compounds containing only one sulfur-oxygen bond are collected.

All compounds containing the —SO_2— grouping have an absorption in the region between 1120 and 1300 cm.$^{-1}$ This region can be tentatively divided into two parts. The first, extending from 1120 to 1160 cm.$^{-1}$, is characteristic of sulfones. The second, comprising the range from 1160 to 1200 cm.$^{-1}$, is characteristic of sulfonic acids, sulfuric acids, and their derivatives.

The second absorption area between 1300 and 1400 cm.$^{-1}$ is somewhat more in doubt. Sulfones definitely have an absorption in the region between 1300 and 1350 cm.$^{-1}$ The absorption of sulfonic acid derivatives in the region 1330 to 1400 cm.$^{-1}$ will have to be investigated further before they can be definitely as-

Table III. Absorption of Compounds Containing One Sulfur-Oxygen Bond

Thionyl chloride		1140	1200[a]	1238	1350
Diphenylsulfoxide	(*13*)	1166[a]	1183		1308
Di-*n*-butylsulfoxide	(*22*)		1190		

[a] Well defined knee in curve at point indicated.

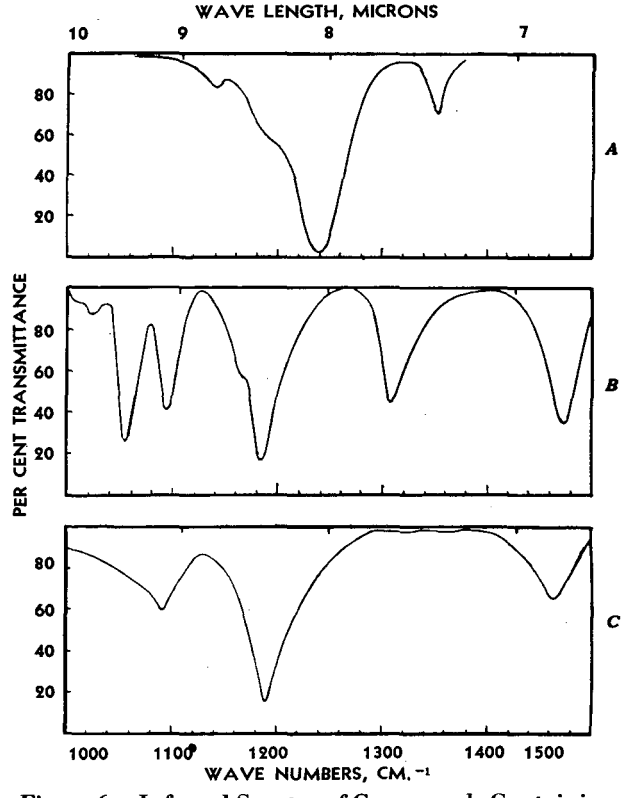

Figure 6. Infrared Spectra of Compounds Containing One Sulfur-Oxygen Bond

A. Thionyl chloride in carbon tetrachloride
B. Diphenylsulfoxide in carbon tetrachloride
C. Di-*n*-butylsulfoxide in carbon tetrachloride

signed to the absorption of the sulfonyl grouping. The disubstituted sulfonamides investigated by Adams and Tjepkema (*1*) show in addition to the absorption reported by them, a second band between 1330 and 1350 cm.$^{-1}$ which corresponds to the second absorption band of the sulfonyl group.

The absorption between 1410 and 1420 cm.$^{-1}$ of the sulfates and the single band of the sulfoxides at 1190 cm.$^{-1}$ need further confirmation.

ACKNOWLEDGMENT

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Punched Card Code for X-Ray Diffraction Powder Data

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A punched card is described which would enable a rapid and exhaustive search to be made of powder x-ray diffraction data for the identification of crystalline chemical compounds. This search could be based on the most intense lines of the x-ray diffraction pattern or on one intense line of the diffraction pattern and the elemental chemical composition of the substance.

THE identification of a chemical compound by the use of x-ray diffraction powder patterns is based on a thesis of Hull (*6*) "that every crystalline substance gives a diffraction pattern; and that the same substance always gives the same pattern." It remained to be shown by Hanawalt and Rinn (*5*) that these patterns were sufficiently different to become the basis of a practical method of analysis. These authors described a method of tabulating powder patterns in a manner suitable for routine chemical identification. This scheme used a large ledger which was unsuitable for reproduction and general distribution. As an alternative, a card file of these data, using basically the same scheme, was published by the American Society for Testing Materials (*1*). This provides an expandable file which is suitable for indexing a comparatively small number of data. The present file, which lists about 4000 substances, has already proved somewhat unwieldy.

If data on three times this number of substances were considered, the size of the file would be such that a search would be very difficult. Alternative solutions to this problem, including the use of punched cards, were discussed in a previous paper (*7*). The present paper presents a revised punched card code which would facilitate the search of a file of powder diffraction data.

The method of Hanawalt and Rinn (*5*) used the three *most* intense (strongest) lines of the powder diffraction pattern as the index lines. The use of three lines is required because variations in x-ray technique and texture of the sample of a given substance cause variations in the relative intensities of the lines of the diffraction pattern. In the ten years this method has been in general use, the use of three index lines has proved necessary and effective for searching purposes.

In searching a file of data for the identification of a powder diffraction pattern, it is usual to start with two diffraction lines of the pattern. Ideally these would be the strongest and second strongest lines of the pattern, but because of the variation in relative intensity of the lines, and the danger that strong lines may be missed in the case of mixtures, an index to be used for an exhaustive search should list the six combinations of the three strongest lines. The card file of diffraction data published by the A.S.T.M. provided three cards for each entry, placed in the index in positions determined by three combinations of the strongest lines—1st, 2nd; 2nd, 1st; 3rd, 1st. The combination 1st, 3rd, which has a relatively high probability of occurrence, and the two combinations 2nd, 3rd and 3rd, 2nd, which become important if the first line were missed, would have required three more cards in

the index. This would have resulted in a file twice the size of the present card index.

A preferred arrangement for the routine search of the card file was described in the foreword to the original set of diffraction data index cards (*1*). This divided the cards into groups or blocks, and each of the three cards for a given substance was placed in the block determined by the three strongest lines of the pattern. Within the block, the cards were arranged in order of the next strongest line of the substance. This placed the cards in the three most probable places, in the card index.

When a punched card system of indexing these data was considered, it was at first thought that the use of the three cards could be eliminated by coding each of the three lines on a single card. This is possible but would have the great disadvantage that a search of the whole file (say 10,000 cards) would have to be made each time a search was attempted. A greatly improved system would be to retain the three-card system, coding the three lines on each of the three cards, and then filing the cards in blocks as described above. With this arrangement, any of the three strongest lines can be made the basis of an exhaustive search by examining one block (approximately 300 cards for 10,000 substances) or at the most two blocks, if the line in question were near the division between two blocks. This would apply equally well if one started with one line or with any two of the three strongest lines. The fact that punched cards can be sorted mechanically greatly facilitates such a search. Because all cards would carry all the data, there would be no necessity, as in the present card index (*1*), to refer to a master or "first" card.

CHEMICAL DATA

The use of chemical composition as a guide to the identification of powder patterns has been emphasized by several publications. Frevel (*4*) described a scheme for the listing of powder data by chemical composition. No determinative tables of this type have been published, possibly because of the difficulty of expanding such tables as new data become available.

With the punched card described above, the chemical composition may be coded in such a manner that it may be used in a search of the diffraction data. This would make possible a search based on the following information: If the unknown is a compound of magnesium having a strong powder line at 3.03 Å., all index cards having data on compounds containing magnesium can be mechanically sorted from the block 3.00 to 3.05. This accomplishes an exhaustive search which otherwise would have required special tables or have been extremely laborious. In a similar manner information on specific subjects such as organic compounds, metals and alloys, minerals, etc., can be readily sorted from the block by use of a code, punched in the card.

THE PUNCHED CARD

The card presented in Figure 1 is designed around the present 3 × 5 inch card published by the American Society for Testing Materials (*1*).

The 3 × 5 inch area in the center of the card gives crystallographic data on the substance sodium chloride. The 0.5-inch margin carries a double row of holes for punched card coding. The coding of the card is described starting at the upper left-hand corner and proceeding clockwise around the card. On the first card, the block code is determined by the first line; the range 2.80 to 2.85 was numbered 40 in a previous description (*7*). This is coded 4 in the tens field (4 deep punch) and zero in the units (no punch). The second line is coded 1 in the units field (1 deep) and 9 in the tenths field (7 and 2 shallow) and +7 in the hundredths field. This number has one zero (01.99). It is the first card. The third line is coded 1.62. The chemical composition code for sodium is 1 deep 3 shallow; for chlorine 11 deep and 3 shallow. The areas *a*, *b*, *c*, and *d* are free for coding other data on the cards.

The margin on these cards could be increased to provide a 4 × 6 inch card with 0.5-inch margins, punched with a double row of holes and printed to give cards similar to that shown in Figure 1.

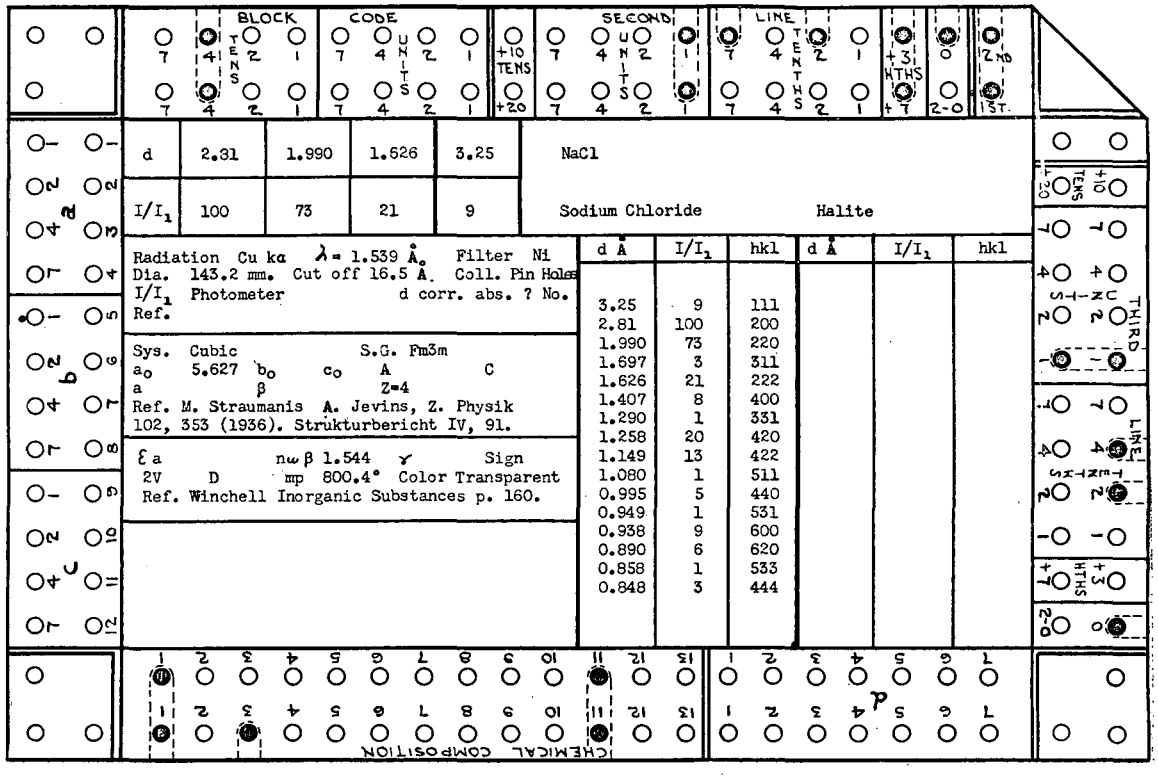

Figure 1. X-Ray Diffraction Data on Punched Card

This type of card is sold under the trade name Keysort by the McBee Company, Athens, Ohio, and under the trade name Cope-Chat by the Copeland Chatterton Company in Canada and England.

The code should be such that it may be readily used in a wide variety of chemical and crystallographic investigations and a part of the card should be left for individual use. The code suggested would use three of each card in the set, designated as first, second, and third cards. On the first card the order of the lines would be coded in the order: strongest, second strongest; on the second card: second strongest, strongest; and on the third card: third strongest, strongest. The designation of the card could be indicated by punches in the upper right-hand corner, by a narrow colored border, or by the color on the card stock.

POWDER LINE CODE

On the first card the strongest line would determine the block. Provision is made for 99 blocks (the Hanawalt index used 78 blocks). The range of these blocks should be made so that an approximately equal number of cards would fall in each. Normally the cards would be kept in the block. The first line code would enable the cards to be sorted into blocks and would give a proof of file, by which misplaced cards could be readily traced. The order within the block would normally be that of the second line. Searches based on third line, chemical composition, innermost line, or some other feature would destroy this order. When such a search is complete, however, the cards should be mechanically sorted back to the second-line sequence for refiling.

A general rule for any card file may be stated as follows: If the data are approached from one point of view more frequently than another, the cards should be kept in that order, and re-sorted to that order for filing. The attitude that punched cards can be sorted mechanically, and therefore no attempt should be made to keep them in any order, is often, in the end, more time-consuming.

Papers on punched card technique by Casey, Bailey, and Cox (*2*, *3*) describe a variety of coding methods. The numerical code used here is the 7-4-2-1 condensed code, in which any of the digits from 1 to 9 can be represented by one of the four digits or a combination of two. When a single row of holes is used, this code is not selective—i.e., 7's cannot be selected from 8 and 9, which are codes 7 + 1 and 7 + 2. The code is made selective with a double row of holes, by punching the digit deep when the digits 7, 4, 2, or 1 are intended. Digits formed by a combination of two are punched shallow. When used in this manner, any digit can be selected from the others. The second and third lines are coded in the actual value in Ångströms, so that it is unnecessary to consult a table. (With the first-line code, the use of a table is no disadvantage, as the field is not ordinarily used for searching.)

Powder lines in Ångström values may be expressed as four-figure numbers—tens, units, tenths, and hundredths. Because the index lines of a powder pattern rarely have values greater than 20, no provision is made for a tens field other than +10 and +20. The units and tenths follow the 7-4-2-1 condensed code and in the hundredths field the cards are coded +3 and +7 when values in this range are given. In this code no provision is made for coding zero and in order to separate readily the 2.0 from the 12.0 and from digits other than zero in the tenths field it is necessary to code the number of zeros in the number. For this purpose zeros in the tens field have to be considered—i.e., 2.0 is considered as having 2 zeros: (02.0). Zeros in the hundredths field are not considered.

With a direct code of the type described, the range selected from a block can be as wide or as narrow as the circumstances indicate—i.e., on a search at 3.50, the cards from 3.0 to 4.0 may be selected, or only those of the narrow range 3.50 to 3.53.

Some consideration should be given to limiting the range of index lines by a rule of the following type. The index lines of a pattern are the three strongest lines between the d values of 1 and 10 Ångströms. This would avoid the difficulty that the range of d values recorded varies with camera construction and radiation employed. This is particularly true of focusing cameras. It would also limit the range over which a spectrometer should be run for identification purposes. The coding of data would also be simplified by such a rule.

CHEMICAL COMPOSITION

A number of schemes for coding chemical composition have been considered (*7*). The scheme suggested here divides the atomic table into thirteen chemically related groups of elements, each of which is given a number which is coded with a deep punch. Within the group, elements are given a second number which is coded with a shallow punch. A suggested code is given in Tables I and II. As each compound contains a number of elements (present average approximately 3.5) which must be coded in this

Table I. Suggested Code

Li	1-2	Fe	5-2	N	9-4	Tm	12-3
Na	1-3	Co	5-3	P	9-5	Yb	12-4
K	1-4	Ni	5-4	As	9-6	Lu	12-5
Rb	1-5	Cu	5-6	Sb	9-7		
Cs	1-6	Ag	5-7	Bi	9-8		
		Au	5-8				
				O	10-9	Ac	13-6
Be	2-8	Ra	6-9	S	10-11	Th	13-7
Mg	2-9	Rh	6-10	Se	10-12	Pa	13-8
Ca	2-10	Pd	6-11	Te	10-13	U	13-9
Sr	2-11	Os	6-12	Po	10-1	Np	13-10
Ba	2-12	Ir	6-13			Pu	13-11
Ra	2-13	Pt	6-1	F	11-2	Am	13-12
				Cl	11-3	Cm	13-1
B	3-1	C	7-2	Br	11-4		
Al	3-2	Si	7-3	I	11-5		
Sc	3-4	Ti	7-4				
Y	3-5	Zr	7-5				
Ga	3-6	Hf	7-6	La	12-6		
In	3-7			Ce	12-7		
Tl	3-8	V	8-7	Pr	12-8		
		Cr	8-9	Nd	12-9		
Zn	4-9	Mn	8-10	Il	12-10		
Cd	4-10	Cb	8-11	Sm	12-11		
Hg	4-11	Mo	8-12	Eu	12-13		
Ge	4-12	Ti	8-13	Gd	12-1		
Sn	4-13	Ta	8-1	Tb	12-1		
Pb	4-1	W	8-2	Dy	12-2		
		Re	8-3	Ho	12-2		
				Er	12-3		

Table II. Suggested Code

Aluminum	Al	3-2	Neodymium	Nd	12-9
Americium	Am	13-12	Neptunium	Np	13-10
Antimony	Sb	9-7	Nickel	Ni	5-4
Arsenic	As	9-6	Nitrogen	N	9-4
Barium	Ba	2-12	Osmium	Os	6-12
Beryllium	Be	2-8	Oxygen	O	10-9
Bismuth	Bi	9-8	Palladium	Pd	6-11
Boron	B	3-1	Phosphorus	P	9-5
Bromine	Br	11-4	Platinum	Pt	6-1
Cadmium	Cd	4-10	Plutonium	Pu	13-11
Calcium	Ca	2-10	Polonium	Po	10-1
Carbon	C	7-2	Potassium	K	1-4
Cerium	Ce	12-7	Praseodymium	Pr	12-8
Cesium	Cs	1-6	Protactinium	Pa	13-8
Chlorine	Cl	11-3	Radium	Ra	2-13
Chromium	Cr	8-9	Rhenium	Re	8-3
Cobalt	Co	5-3	Rhodium	Rh	6-10
Columbium	Cb	8-11	Rubidium	Rb	1-5
Copper	Cu	5-6	Ruthenium	Ru	6-9
Curium	Cm	13-1	Samarium	Sm	12-11
Dysprosium	Dy	12-2	Scandium	Sc	3-4
Erbium	Er	12-3	Selenium	Se	10-12
Europium	Eu	12-13	Silicon	Si	7-3
Fluorine	F	11-2	Silver	Ag	5-7
Gadolinium	Gd	12-1	Sodium	Na	1-3
Gallium	Ga	3-6	Strontium	Sr	2-11
Germanium	Ge	4-12	Sulfur	S	10-11
Gold	Au	5-11	Tantalum	Ta	8-1
Hafnium	Hf	7-6	Tellurium	Te	10-13
Holmium	Ho	12-2	Terbium	Tb	12-1
Illinium	Il	12-10	Thallium	Tl	3-8
Indium	In	3-7	Thorium	Th	13-7
Iodine	I	11-5	Thulium	Tm	12-3
Iridium	Ir	6-13	Tin	Sn	4-13
Iron	Fe	5-2	Titanium	Ti	8-13
Lanthanum	La	12-6	Tungsten	W	8-2
Lead	Pb	4-1	Uranium	U	13-9
Lithium	Li	1-2	Vanadium	V	8-7
Lutecium	Lu	12-5	Ytterbium	Yb	12-4
Magnesium	Mg	2-9	Yttrium	Y	3-5
Manganese	Mn	8-10	Zinc	Zn	4-9
Mercury	Hg	4-11	Zirconium	Zr	7-5
Molybdenum	Mo	8-12			

field, there will be some overlapping where a particular element is selected by use of this code. For instance, when compounds of calcium are selected, in addition to all the cards having data on compounds containing calcium, other cards will fall. The deeply punched code number for calcium, however, assures that these additional cards will contain elements chemically related to calcium and will form only a small proportion of the cards sorted from the block. This difficulty could be overcome, in part, by extending the code to a larger number of groups (from 13 to 20 groups) or by using a three-number code for each element (one deep punch and two shallow). In its present form, it is felt that this difficulty would not for practical purposes be serious. Certain arbitrary rules could be set up to simplify this code—e.g., oxygen is coded in oxides only, carbon is coded in inorganic compounds only. If in a laboratory some elements were of particular interest and the overlapping mentioned above is undesirable, these elements could be given a direct code in the spare parts of the card. In the spare parts of the card organic compounds, metal-organic compounds, alloys, minerals, etc., could also be given a direct code. Other codes based on melting point, optical properties, innermost lines of the diffraction pattern, or other easily measured determinative property of a substance could be developed.

CONCLUSION

The publication of powder diffraction data on punched cards of the type described would increase the cost of publication, but this increase should be justified if the usefulness of the index were increased by making the data more available. The proposed code is only one of many that could be developed; the individual requirements of workers would necessitate variations of the method employed, and many of these variations would make use of the spare parts of the card.

ACKNOWLEDGMENT

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Determination of Pyridine and Its Homologs in Hydrocarbons by Ultraviolet Spectrophotometry

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Pyridine may be determined in samples of hydrocarbons of the kerosene-naphtha range rapidly and by a relatively simple technique. The method may be extended to include quinoline and its homologs. In its application to nitrogen bases, presumably pyridine homologs, by measurement of ultraviolet absorption at 270 mμ, interference from phenol is serious.

THE characteristics of pyridine, quinoline, and their homologs in absorption of ultraviolet radiant energy are well known as evidenced by the many publications of their spectra. Some of their spectrograms have been issued by A.P.I. Research Project 44 (*1*). An examination of the ultraviolet absorption spectra of pyridine and quinoline (Figure 1) reveals their propitious nature for analytical spectrophotometry on two counts: The absorption maximum of each compound is steep and narrow, and each maximum occurs where the other shows a minimum or no absorption at all. That pyridine, in solutions of dilute inorganic acid, can be determined by spectrophotometric means appears reasonable; over the range of 1 to 25 p.p.m., the absorbances at 250 mμ of standard pyridine solutions in dilute sulfuric acid bear a linear relationship to their concentrations.

A search of *Chemical Abstracts* from 1907 to August 10, 1948, revealed only one analytical method for pyridine (and quinoline) based on ultraviolet spectrophotometry: the fairly recent method of Hofmann (*2*). In this method quantities of pyridine as small as 0.01 mg. per liter in air samples were absorbed in 1 *N* sulfuric acid, following which the pyridine was determined by ultraviolet absorption at 255 mμ. The method reported herein bears a similarity to Hofmann's; however, it was developed prior to any knowledge of the existence of Hofmann's method.

In order to develop a rapid method for the analysis of pyridine and quinoline compounds, known to be present in California naphthas, ultraviolet spectrophotometry was tried. Pyridine and some of its methyl-substituted compounds showed absorption peaks in the region from 250 to 270 mμ (*1*). Spectrophotometric analyses for such constituents could not be applied directly to refinery samples of gasoline, naphtha, and kerosene, inasmuch as they may contain varying amounts of aromatic hydrocarbons which show strong absorption in the region extending approximately from 240 to 280 mμ.

OUTLINE OF METHOD

Pyridine, a weakly basic substance, is readily extracted with dilute phosphoric acid from hydrocarbons such as naphtha, gasoline, and kerosene. The absorbance (optical density) of that extract, or a suitable dilution, is measured at 255 mμ. The value obtained is converted to concentration of pyridine by reference to a standard graph. From that concentration its content in the original sample is readily calculated.

EXPERIMENTAL

Apparatus and Reagents. Beckman quartz spectrophotometer, Model DU (or equivalent instrument), fully equipped.

Separatory funnels, 125 ml.

Volumetric flasks, glass-stoppered, of various sizes.

Volumetric pipets of various sizes.

Phosphoric acid (H_3PO_4), 10% by weight in aqueous solution.

Calibration of Instrument. A master solution of pyridine (colorless, for Karl Fischer reagent, 214-H, Eastman Kodak Company) was prepared by dissolving 0.1320 gram in enough 10% by weight phosphoric acid to make 1 liter of solution. Dilutions ranging from 0.0013 to 0.0264 gram of pyridine per liter of solution were prepared from that master solution, using distilled water as the diluent. The determination of the absorption characteristics on the dilution consisting of 0.0132 gram of pyridine per liter revealed an absorption maximum at 255 mμ. Accordingly, absorbance measurements were made on the several dilutions at 255 mμ, using distilled water in the reference cell. In the dilutions measured the phosphoric acid content varied from 0.1 to 2%, in which range it possessed negligible ultraviolet absorption. After applying absorption cell corrections to the observed absorbances, the values obtained were plotted against concentration. The resultant graph is reproduced in Figure 2.

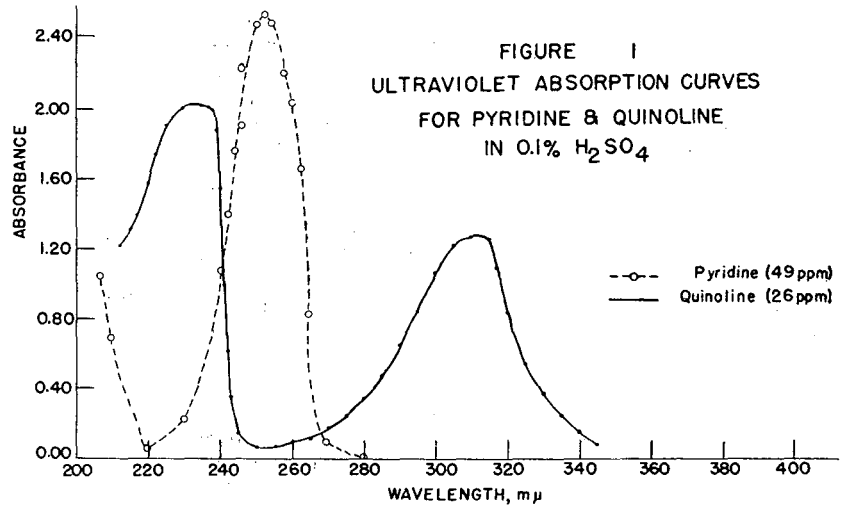

FIGURE 1
ULTRAVIOLET ABSORPTION CURVES
FOR PYRIDINE & QUINOLINE
IN 0.1% H_2SO_4

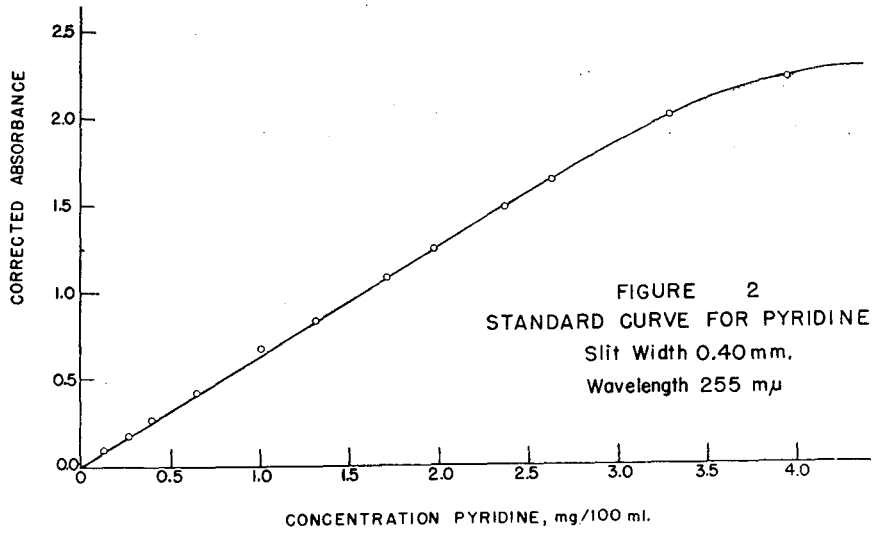

FIGURE 2
STANDARD CURVE FOR PYRIDINE
Slit Width 0.40 mm.
Wavelength 255 mμ

Extraction of Pyridine from Spectroscopic Solvent. To determine if pyridine could be extracted quantitatively by dilute phosphoric acid from hydrocarbons such as naphtha, gasoline, and kerosene, the following work was done:

Using 50-ml. portions, six different concentrations of pyridine in spectroscopic solvent (a highly purified pentylene alkylate, probably chiefly octanes) were extracted one to three times with 25-ml. portions of 10% by weight phosphoric acid. Each acidic extract was withdrawn and saved separately. The first extractions were made by shaking for 2 minutes; succeeding extractions were shaken for 1 minute. Each acidic extract was made to definite volume and thoroughly shaken. A blank consisting of 50 ml. of spectroscopic solvent only was similarly treated. The extracts were analyzed for their pyridine contents by measuring their absorbances, and readings, corrected for absorption cell variations and the blank, were converted to pyridine concentration using the standard graph shown in Figure 2. A summary of the data obtained is given in Table I.

Table I. Extraction of Pyridine from Spectroscopic Solvent Solutions with 10% Phosphoric Acid

Sample	Pyridine Content	Pyridine Found 1st extract	2nd extract	3rd extract	Pyridine Recovered, 1st Extract
	P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	%
A	100	100.6	0.2	0.2	100.6
		102.2			102.2
		102.4			102.4
B	70	70.0	0.4	0	100.0
		73.0			104.3
		71.0			101.4
C	50	50.0	0	0	100.0
		50.5			101.0
		52.5			105.0
	30	31.5	0	0	105.0
		30.3			101.0
		30.5			101.7
E	20	19.6	0	0	98.0
		20.7			103.5
		20.1[a]			100.5
F	10	9.1	0	0	91.0
		10.1			101.0
		10.1			101.0

[a] Basis, extraction of 20-ml. instead of 50-ml. portion.

The ready and quantitative extraction of pyridine from spectroscopic solvent in one extraction with 10% by weight phosphoric acid is evident from Table I. For that reason only one extract is shown on the replicate determinations. The consistently higher values found in the repeated first extractions may have resulted from a lower room temperature prevailing during the pipetting of the replicate samples.

PROCEDURE

For samples containing 0.15 gram or less of pyridine per 100 ml., pipet a 50-ml. portion into a 125-ml. separatory funnel. For samples having higher concentrations of pyridine, weigh a portion containing not more than 75 mg. of pyridine into a 125-ml. separatory funnel holding 50 ml. of spectroscopic solvent.

Extract the sample (and the blank) twice with 25-ml. portions of 10% by weight phosphoric acid, shaking the first extraction for 2 minutes and the second for 1 minute. Withdraw the lower, acid layer after each extraction into a 100-ml. glass-stoppered volumetric flask. Make the combined acidic extracts to the mark with distilled water, and mix thoroughly.

If the combined and diluted extracts contain not more than 1.5 mg. of pyridine per 100 ml., absorbance may be measured. If otherwise, dilute further. In analyzing solutions of unknown strength, the need for further dilution is indicated if the absorbance (optical density) measures more than 1.0, or at the most 1.5.

Transfer a portion of the final dilution to a 10-mm. silica absorption cell. Measure its absorbance, after setting the reference cell filled with distilled water to read 100% transmittance, at a wave length of 255 mμ and a slit width of 0.40 mm. (The exact values of the wave length and the slit width vary slightly among spectrophotometers.) Correct the absorbance observed, if necessary, for the absorption cell correction and the absorbance of the blank.

Using the standard graph (Figure 2), prepared in the calibration of the instrument, convert the corrected absorbance to its equivalent concentration of pyridine. With that information, the pyridine content may be calculated from these formulas:

$$\text{Pyridine content, mg. per 100 ml.} = \frac{C \times F \times 100}{V}$$

$$\text{Pyridine content, wt. \%} = \frac{C \times F \times 100}{W \times 1000}$$

where

C = concentration (mg. per 100 ml.) of pyridine read from standard graph (Figure 2)
F = dilution factor or ratio
V = volume of sample taken in milliliters
W = weight of sample taken in grams

OTHER APPLICATIONS

The suitability of pyridine for determination by ultraviolet spectrophotometry suggested that its homologs, as well as quinoline and its homologs, might likewise be so determined. The method actually was used to determine basic nitrogenous compounds (nitrogen bases) in California naphtha. No attempt was made to establish their chemical nature. The fact that they absorbed maximally at 270 mμ and showed no absorption at 310 mμ (where quinoline absorbs) indicated the possibility of their being pyridine homologs. Work done by the Union Oil Company on the ultraviolet absorption of methylpyridines, as shown in several spectrograms issued by A.P.I. Research Project 44 (*1*), indicated a shift in maxima toward longer wave lengths as the result of methyl substitution on the pyridine ring.

Inasmuch as the chemical identity of the nitrogen bases was not established, and they probably consisted of mixtures, somewhat empirical means were necessary in preparing a standard graph for analytical purposes. Exhaustive acid extractions of several large samples of California naphtha were made and the combined extracts served as the master solution of nitrogen bases. Its concentration was determined by isolating and weighing the nitrogen bases contained in an aliquot portion. A standard graph like that for pyridine was then made.

Even though the procedure for nitrogen bases gave relative rather than absolute values, useful results were obtained. It was realized, however, that nonnitrogenous acid-extractable compounds might be included as nitrogen bases. An analysis for nitrogen was not made on the nitrogen bases isolated, partly because at that time it was thought that no satisfactory method existed. For naphthas other than the stock studied, it would be necessary to prepare a separate standard graph, for the absorption characteristics of the nitrogen bases may vary from stock to stock.

INTERFERENCES

Logically, any substance extractable from hydrocarbon solutions by dilute phosphoric acid and possessing an absorption for ultraviolet energy at 255 mμ will interfere in the determination of pyridine by the described method. An investigation of substances possessing those requisites was not made. In the application of the procedure to the analysis of nitrogen bases contained in California naphtha, however, the serious interference of phenol was discovered. It was found that phenol was extractable from its solution in spectroscopic solvent with 10% by weight phosphoric acid; Seidell (*3*) gave the solubility of phenol as 4% in 25% by weight phosphoric acid (H_3PO_4), increasing with decreasing strength of the acid.

Occasionally, in determining nitrogen bases in California naphtha, a solid substance formed at the interface of the naphtha and acid layers. Its nature was not known; however, an alcoholic solution of that solid exhibited a spectrogram similar to that for the nitrogen bases. Because of the strong likelihood that such solids were or contained nitrogen bases, means to reduce or prevent their occurrence should be used. A considerable reduction in the size of sample taken proved very helpful in the one instance tried.

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Permission of the management of The Texas Company to release the information embodied herein is gratefully acknowledged. An expression of thanks is also due W. S. Palmer, L. V. Wike, G. H. Miller, T. C. Roddy, Jr., and S. W. Denton for their valuable suggestions offered in the course of the investigation. The kind assistance of Elken Gibson in preparing both graphs is gratefully acknowledged.

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SURFACE TENSION MEASUREMENT

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IN THE latter part of 1946, subsequent to the development of the Interchemical inclined tube viscometer (*2*), it was observed that the conversion factor developed to convert time in seconds for the bubble to flow over a given distance in a tube, to kinematic viscosity was not exactly the same for all materials measured. Upon investigation of this phenomenon, it was postulated that the variation was probably due to surface tension. Barr (*1*) has shown that surface tension is a definite factor influencing the rate at which an air bubble will move through a tube held in a vertical position. There is no reason to believe, therefore, that surface tension would not also be a factor when the tube is held in a position slightly inclined from the horizontal.

The difference in the horizontal bubble lengths (when the volume of the bubble is held constant) of materials having a considerable range of surface tension indicated that the measurement of this bubble length might very well afford a very simple means of determining surface tension. Preliminary investigations were initiated to determine the ratio of the height of the air space above the liquid in a tube, which is the controlling facter for the volume of the air bubble in a tube of given diameter, and the length of the static bubble when the tube is placed in a horizontal position. Earlier data indicated that this ratio was approximately constant over a sizable range of bubble volume. However, a more accurate and detailed study recently conducted has shown that this ratio

By determining the length of an air bubble of given volume in a horizontal tube of known diameter, the surface tension of the material in the tube may be determined. This method is particularly suited for measuring surface tension of viscous materials on which the usual methods of measurement fail (4).

is not constant. Therefore, for all subsequent work, the height of the air space above the bubble, and thus the volume of the bubble, was held constant. The effect of the size of tubes was also investigated, and it was concluded that the smaller the tube, the more pronounced was the effect of surface tension. This observation is also substantiated by Barr (*1*).

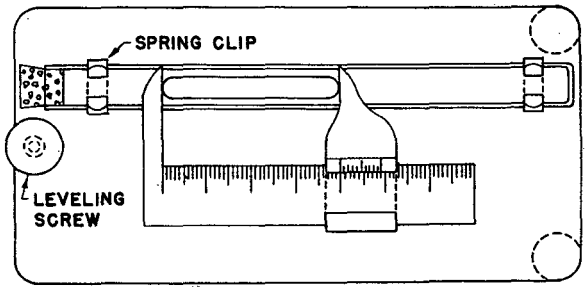


Figure 1. Top View of Surface Tension Apparatus

The tube size finally selected for all subsequent work was a compromise which offered a fair range of difference in bubble length with respect to surface tension, and still afforded a tube large enough to be readily filled with viscous materials. The tubes chosen were those currently being used with the Interchemical inclined tube viscometer (*2*).

APPARATUS

Inasmuch as it was desired to substantiate or disprove the postulate at an early date, and no entirely satisfactory method of surface tension measurement was immediately on hand which could be used for obtaining accurate check values on materials to be considered, eighteen chemically pure liquids were obtained whose surface tension values were known (*3*).

The glass tubes used for the investigation were approximately 12.5 cm. long and 0.8 cm. in inside diameter. An apparatus was developed which would conveniently permit the leveling of these tubes in order to hold the air bubble in a static position and, at the same time, afford an accurate means of measurement of the horizontal bubble length. A drawing of this apparatus is shown in Figure 1.

RESULTS OBTAINED

Table I contains data that were compiled on the eighteen known materials. The air space for each material when the tube was in a vertical position was held constant at approximately 1.00 cm. The shortest bubble length under these conditions was 1.79 cm. and the longest was 4.22 cm. Thus, a sufficient spread is obtained to afford reasonable accuracy over the entire range of surface tension. It has been shown that if the volume of the air bubble is increased, there is also an increase in the difference in bubble lengths observed. Thus by designing the apparatus to accommodate the use of longer bubbles, the accuracy of this method can be improved.

A considerable amount of effort has been expended in attempting to derive a mathematical relationship between horizontal bubble length and surface tension. In the attempted mathematical analysis, not only the volume of the bubble and horizontal bubble lengths have been considered, but also the shape of the bubble, density of the material in the tube, and diameter of the tube. All the results obtained by these attempts at mathematical analysis have given a definite, positive correlation of the horizontal bubble length to surface tension, but, even so, the results obtained have not been so acceptable as the empirical relationship that has been developed.

Considering the dimensions of the tube to be held constant and disregarding other factors that might possibly influence the results, it can be said that the density of the material in a hori-

Table I. Horizontal Bubble Length Observed on Eighteen Materials

(Volume of bubble held constant)

Material	Horizontal Bubble Length, Cm.	Material	Horizontal Bubble Length, Cm.
Ether	3.42	Benzene	2.57
Acetaldehyde	3.05	Carbon disulfide	3.18
Methyl alcohol	2.98	Ethylene bromide	3.94
Ethyl acetate	3.04	Benzyl alcohol	2.41
Butyl alcohol	2.77	Furfural	2.40
Methyl ethyl ketone	2.85	Ethylene glycol	2.26
Methyl acetate	3.04	Formamide	2.10
Carbon tetrachloride	4.22	Glycerol	2.20
Acetic acid	3.13	Water	1.79

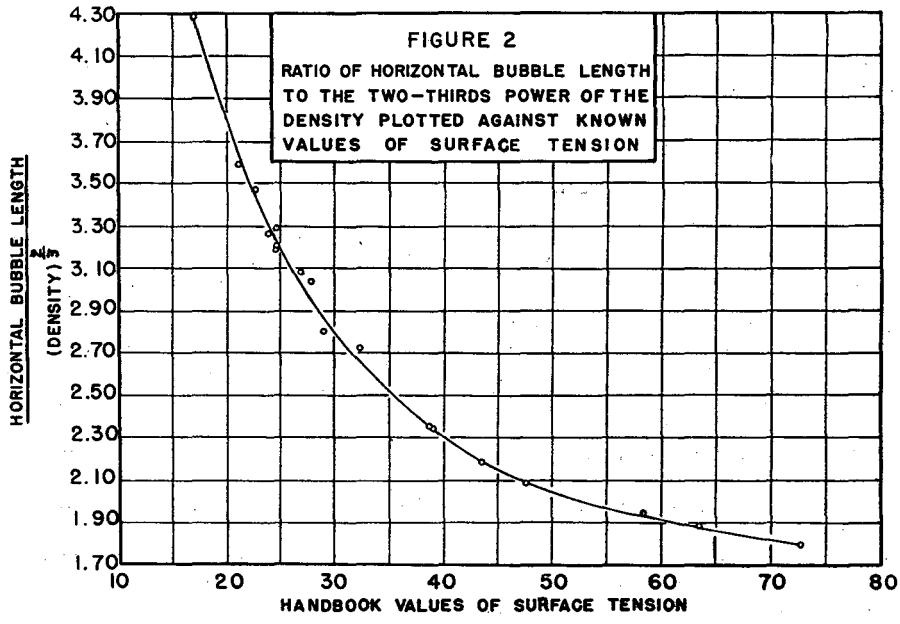
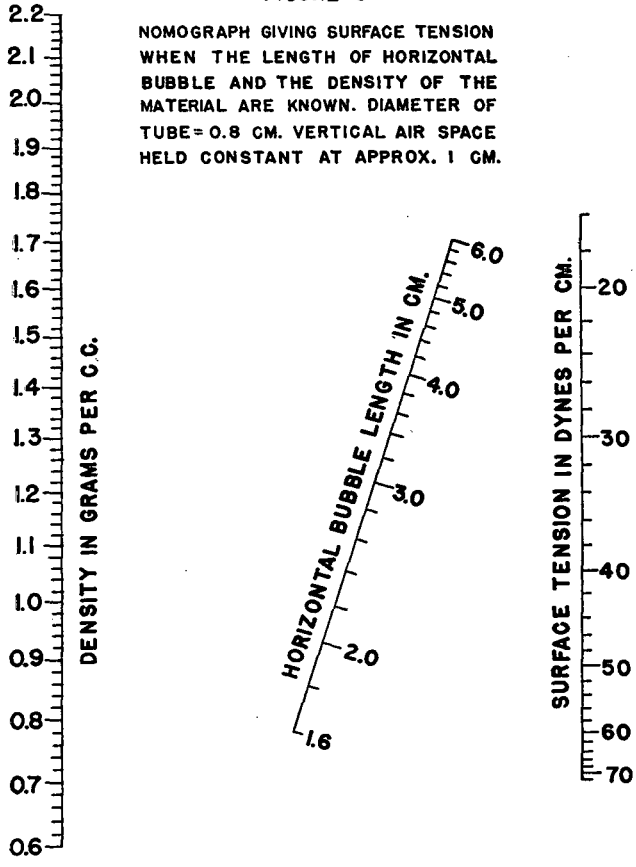


Table II. Surface Tension Values

Material	Density	Check Values (3)	Values from Figure 2	Error	Calculated by Formula	Error
	G./cc.	*Dynes/cm.*	*Dynes/cm.*	%	*Dynes/cm.*	%
Ether	0.714	17.0	17.0	0	17.5	+2.9
Acetaldehyde	0.783	21.2	21.5	+1.4	21.3	+0.5
Methyl alcohol	0.796	22.6	22.4	−0.9	22.2	−1.8
Ethyl acetate	0.901	23.9	24.3	+1.7	24.0	−0.4
Butyl alcohol	0.810	24.6	25.0	+1.7	24.7	+0.4
Methyl ethyl ketone	0.805	24.6	24.0	−2.4	23.7	−3.7
Methyl acetate	0.927	24.6	24.8	+0.8	24.6	0
Carbon tetrachloride	1.60	27.0	26.3	−2.6	25.9	−4.1
Acetic acid	1.05	27.8	26.8	−3.6	27.3	−1.8
Benzene	0.879	28.9	30.0	+3.7	29.6	+2.4
Carbon disulfide	1.26	32.3	31.4	−2.8	30.9	−4.3
Ethylene bromide	2.17	38.8	38.8	0	39.3	+1.3
Benzyl alcohol	1.05	39.0	39.0	0	39.6	+1.5
Furfural	1.16	43.5	43.5	0	45.2	+3.9
Ethylene glycol	1.12	47.7	48.0	+0.6	49.2	+3.1
Formamide	1.13	58.2	57.6	−1.0	57.8	−0.7
Glycerol	1.26	63.4	62.8	−0.9	62.3	−1.7
Water	1.00	72.8	72.4	−0	70.7	−2.7
Average error				1.3		2.2
Maximum error				3.7		4.3

FIGURE 3

NOMOGRAPH GIVING SURFACE TENSION WHEN THE LENGTH OF HORIZONTAL BUBBLE AND THE DENSITY OF THE MATERIAL ARE KNOWN. DIAMETER OF TUBE = 0.8 CM. VERTICAL AIR SPACE HELD CONSTANT AT APPROX. 1 CM.



zontal tube has a tendency to elongate the bubble, whereas the surface tension acts in the opposite direction to shorten the bubble. Thus the surface tension is directly proportional to some function of the density of the material surrounding the bubble, and inversely proportional to some function of the length of the bubble. It was determined empirically that when the bubble volume was held constant, and the ratio of the horizontal bubble length to the two thirds power of density was plotted against the known values of surface tension, a smooth curve was obtained, as is shown in Figure 2. Table II shows the density of the materials used as well as the surface tension values taken from the literature (3) and those obtained by use of the method described and the graph shown in Figure 2. The difference between the experimental and known values were very small, as indicated for each of the materials in Table II.

By deriving an approximate mathematical expression for the curve shown in Figure 2, it is possible to solve for surface tension directly. This equation is:

$$S = \frac{42}{L/d^{2/3} - 1.16} + 4$$

where S = surface tension in dynes per centimeter, L = length of horizontal bubble in centimeters, and d = density of material in grams per cubic centimeter.

Table II also shows the values of surface tension obtained for each material by the use of this formula, which is valid only for the particular tube diameter and bubble volume used.

A nomograph, shown in Figure 3, was constructed as an aid for determining surface tension by this method. By the use of this nomograph it is possible to determine surface tension directly by simply placing a straightedge across the observed values of density and horizontal bubble length. This nomograph was constructed from the curve shown in Figure 2 and is valid only for the particular size of tube and bubble volume used in this work.

Several factors are recognized as influencing the values of surface tension that were obtained by this method. Probably the most serious is temperature. All experimental work for the data presented was conducted at room temperature, which varied from 70° to 80° F., while the check values used were given at 68° F. The tubes possessed slight inherent variations in diameter, which may have resulted in slight errors in the data obtained. The closures of the tubes left much to be desired. Cork stoppers were used, which made it difficult to ensure constant air space pressure and to determine accurately the air space in the tube when held vertically. These cork stoppers may also have been a source of contamination.

The data presented were obtained from the first exploratory investigation, which was conducted for the purpose of establishing the fact that surface tension did influence viscosity readings in instruments of bubble tube type. For this reason, close controls normally exercised in research work were not imposed. Because of exigencies of other matters, the authors have not been able to conduct a more fundamental investigation of the phenomenon described. The importance of such factors as contact angle, optimum tube dimensions, etc., is acknowledged, though additional work would be required to consider these factors rigorously

CONCLUSIONS

From the limited data presented, it appears that the proposed method of surface tension measurement is valid and that a high degree of accuracy is obtained. Accuracy can be further improved by the use of specially designed tubes and improved auxiliary equipment for measuring the height of the air space in the vertical tube and the length of the bubble in the horizontal tube.

The fact that the materials under consideration are contained in closed tubes obviates evaporation and contamination.

It is very easy to make determinations in these tubes under the surface of a liquid temperature control bath. Therefore, it is possible to control temperature very accurately, and to use a very

wide range of temperature conditions for surface tension investigations.

The cost of apparatus required to make determinations on surface tension by this method is nominal.

Because the method reduces to a minimum the chance of human error in making this type of measurement, it is ideally suited for use not only in the research laboratory but by nontechnical personnel in production control laboratories.

The viscosity of the material measured has no apparent effect on surface tension measurement by this method. To date materials have been measured which range in viscosity from approximately 0.01 to 10 poises, and there is no indication that the method will not apply to materials of even much higher viscosity.

ACKNOWLEDGMENT

The observations of Howard Ellerhorst, Jr., in the early work on this method of surface tension measurement are acknowledged, as well as the helpful suggestions and counsel that have been received from W. D. Harkins, E. N. Harvey, Jr., and Loy S. Engle.

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VITAMIN A IN FISH OILS

Relative Merits of Four Methods of Assay

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The various methods of assaying for vitamin A were standardized in terms of the new U. S. P. vitamin A reference standard and then applied to 28 representative fish oils of commerce. The vitamin A content of the nonsaponifiable fraction of these fish oils was determined by the spectrophotometric, antimony trichloride, and glycerol dichlorohydrin methods of assay. In addition, the chromatographed eluate of each fish oil was assayed by the spectrophotometric procedure. The data are presented in tabular and graphic form and are discussed. Much of the extraneous material normally present in some fish oils, which interferes with vitamin A determination by chemicophysical methods, may be at least partially eliminated by saponification and by chromatography without serious loss of the vitamin. In general, the highest vitamin potency was indicated by the spectrophotometric method of assay when applied to the whole oils, whereas the biological method generally indicated the minimum potency. The antimony trichloride and glycerol dichlorohydrin methods of assay, when applied to the nonsaponifiable fraction, yielded vitamin values somewhat more comparable to those obtained by the biological assay than were the values obtained by the spectrophotometric procedure when conversion factors of 2000 and 1925 were used in the calculations.

THE scientific literature (*8*, *13*, *22*, *26*) indicates that no general agreement exists among investigators as to which of the chemophysical methods of estimating vitamin A yields data more nearly comparable to those obtained through bioassay, especially when these methods are applied to a wide range of vitamin A carriers. However, in the past, one of the difficulties most frequently encountered in assaying for vitamin A by these methods has been the lack of a vitamin A standard that possessed the desired stability, specificity, and reproducibility (*14*). Because crystalline vitamin A acetate seems to possess many of the characteristics of a satisfactory vitamin A standard (*6*, *14*), the use of vitamin A acetate as a standard in a critical study of the effectiveness of the existing methods in measuring the vitamin A content of a number of representative commercial fish oils was believed to be of sufficient interest to merit further study. The investigation herein reported involved the standardization of the methods of vitamin A assay on the basis of crystalline vitamin A acetate, application of the standardized methods to various vitamin A carriers in the form of fish oils, and a comparison of the data obtained by the different methods of assay.

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ASSAY METHODS INVESTIGATED

Three chemicophysical methods of analysis were studied—the spectrophotometric, the antimony trichloride, and the glycerol 1,3-dichlorohydrin colorimetric methods. These methods were applied to a series of representative fish oils and to their nonsaponifiable residues. In addition, the spectrophotometric method of assay was applied to eluates of these oils obtained through bone meal chromatography, and the whole oils were assayed biologically for their vitamin A content by the official U.S.P. method.

Spectrophotometric Method. Except where specifically stated, a Beckman quartz spectrophotometer with a hydrogen discharge tube as the source of illumination was used in all spectrophotometric measurements. The details of the technique employed in this phase of the investigation have been described (*6*).

APPLIED TO VITAMIN A ACETATE. It has been reported (*6*, *14*) that vitamin A acetate, either in the crystalline state or when dissolved in certain oils, remains relatively stable during the storage for a reasonable period of time. Furthermore, it was found that the vitamin A content of solutions of this ester in a number of organic solvents, including isopropyl alcohol, remained stable for at least 24 hours. In consequence, vitamin A acetate dissolved in isopropyl alcohol provides a suitable standard for use in the spectrophotometric method for assay for vitamin A.

In employing such a solution as a standard in the present studies, the average $E^{1\%}_{1\text{ cm.}}$–325 mμ (1520) for a number of different batches of crystalline vitamin A acetate when dissolved in isopropyl alcohol (*14*) was used in conjunction with the calculated biological potency of 1 gram of crystalline vitamin A acetate to obtain a conversion factor of 1925. The biological potency of 1 gram of vitamin A acetate had previously been calculated to be 2.926×10^6 U.S.P. units, based on the $E^{1\%}_{1\text{ cm.}}$–328 mμ of 1545 for crystalline vitamin A acetate when dissolved in absolute ethyl alcohol and the use of the conversion factor 1894 (*32*). However, if one chooses to ignore the effect of differences in solvents and the slight effect of differences in wave lengths on spectrophotometric measurements and compare the above vitamin A acetate with that present in the U.S.P. vitamin A reference standard by multiplying the $E^{1\%}_{1\text{ cm.}}$–325 mμ (1520) by the conversion factor 1894, one obtains a calculated biological potency of 2.879×10^6 U.S.P. units per gram. The potency indicated is in reasonably good agreement with the potency calculated for the vitamin A acetate present in the U.S.P. vitamin A reference standard, even though the effect of using a different solvent and a different wave length is ignored in the latter calculation.

Applied to Whole Fish Oil. To determine the vitamin A content of the whole fish oils by the spectrophotometric method, duplicate samples of the oils in amounts based on previously reported vitamin A values were weighed and dissolved in isopropyl alcohol. Extinction coefficients—i.e., $E^{1\%}_{1\text{ cm.}}$–325 mμ—were determined for these solutions, adjustments in concentration being made if the optical densities did not fall within the desired range (*6*). Concomitantly, optical densities of these solutions were determined at other wave lengths in order to draw absorption curves for each oil covering the spectral range of 260 to 370 mμ.

The vitamin A content of each of the above solutions was calculated by multiplying the extinction coefficient at 325 mμ by the conversion factor of 1925.

Applied to Nonsaponifiable Fractions of Fish Oils. The procedure as outlined by Wilkie (*33*) was used to extract the nonsaponifiable fraction of the fish oil preliminary to measuring its vitamin A content. Complete removal of the alkali from the ether solution of the nonsaponifiable matter was checked by testing the aqueous washings with phenolphthalein.

The ether solution of the nonsaponifiable fraction was dried by storage over anhydrous sodium sulfate, quantitatively transferred to a clean flask, and made to volume. Aliquots of the ether extract were evaporated on a steam bath to a few milliliters, and the final traces of ether were removed by means of a current of carbon dioxide. The residue was dissolved in isopropyl alcohol and the vitamin A content was determined spectrophotometrically.

Other aliquots of the ether extract were examined by the antimony trichloride and glycerol dichlorohydrin methods of assay. The effect of saponification on the vitamin A content of the oil is discussed below. Other portions of the ether extracts were evaporated in tared flasks for the purpose of determining the percentage of nonsaponifiable matter in the respective fish oils.

Applied to Chromatographed Fractions of Fish Oils. Bone meal was the only adsorbing agent employed in the chromatographic studies. The bone meal used in these experiments was treated according to the suggestions of Glover *et al.* (*11*) and 5-gram samples were used to prepare 2 × 10 cm. adsorbing columns. The following modification of the Glover procedure was employed in chromatographing the petroleum ether solutions of the whole fish oils.

Solutions containing 1% or less of the vitamin A–bearing oil, in redistilled petroleum ether (35° to 65° C.), were used in order to avoid excess greasing of the bone meal. The bone meal column was first wetted with 15 ml. of petroleum ether, stirred to remove air bubbles, and lightly tamped to ensure uniform compactness. All but a few milliliters of this petroleum ether were allowed to pass through the column and were discarded before the sample was introduced into the column. An aliquot of the petroleum ether–oil solution, containing 3000 U.S.P. units or less of vitamin A, was then added to the column, and subsequently eluted with 50 ml. of acetone and then with 25 ml. of petroleum ether; the washings were discarded and the succeeding oil sample was introduced into the column before the last few milliliters of petroleum ether had passed through the bone meal. All elutions were done by gravity. The petroleum ether was removed from the eluate in the usual manner. The residue was taken up in isopropyl alcohol and the vitamin A content of the solution was determined by the spectrophotometric technique.

Antimony Trichloride Method. The antimony trichloride method of assaying for vitamin A (*5*, Carr-Price reaction) was essentially the procedure of Koehn and Sherman (*18*). The antimony trichloride reagent was prepared by saturating chloroform with antimony trichloride, which had previously been purified in the manner suggested by a British Pharmacopoeia Sub-Commission (*3*). The reagent, when stored in amber glass bottles in the presence of an excess of crystalline antiomny trichloride, was found to be stable for considerable periods, as had been noted by Ellenberger *et al.* (*9*). The Evelyn photoelectric colorimeter, equipped with a 620-mμ filter, was used to determine the optical densities of the resulting colorations.

Applied to Vitamin A Acetate. Vitamin A acetate solutions prepared from the crystalline vitamin and from the vitamin dissolved in a commercially refined and deodorized cottonseed oil (Wesson oil) were employed in preparing calibration curves for use in connection with this colorimetric method of assay. From the standpoint of stability, these solutions had proved satisfactory as they remained stable for at least 48 hours when stored in the dark at 5° C. During intervals of this length, such solutions were warmed to room temperature several times during the course of a series of assays without noticeable loss of the vitamin A. Furthermore, because the calibration curves prepared for use in connection with the two colorimetric methods were found to be essentially straight lines within the limits of the usual concentrations, k values relating the concentration of vitamin A acetate to optical density were calculated. The k values for the antimony trichloride reaction product obtained from calibration curves which had been previously prepared while using a number of different samples of vitamin A acetate showed a variation of less than 2%.

Applied to Unsaponified and Saponified Fish Oils. Duplicate samples of the whole fish oils and their respective nonsaponifiable fractions in amounts based on previously indicated potencies were dissolved in chloroform and the concentrations adjusted to permit the galvanometer readings in the colorimetric measurement of the antimony trichloride reaction product to fall between 30 and 70. Within this range, the optical density of the resultant colors had been found to be a linear function of vitamin A acetate concentration.

By using the k values described above for relating the optical densities of the resulting colors to vitamin A acetate concentration, the vitamin A content of the oils in terms of micrograms of vitamin A acetate were calculated for the oils and their nonsaponifiable fractions. Multiplication of these values by the assigned biological potency of vitamin A acetate as mentioned above gave the vitamin A potency in terms of U.S.P. units per gram of oil.

Although anhydrous C.P. chloroform was generally used as the solvent for the oil samples, it was noted that the chloroform from discarded oil solutions could be reused if recovered by the procedure outlined for the purification of chloroform (*3*). In order to prevent the formation of phosgene in purified chloroform, as noted by Koehn and Sherman (*18*), 0.5% (by volume) of U.S.P. 95% ethyl alcohol was added to the chloroform. Lodi (*19*) has reported that this amount of alcohol does not affect the color values obtained from the reaction of the antimony trichloride reagent

with vitamin A. The chloroform was kept free of moisture by storage over anhydrous sodium sulfate.

Glycerol 1,3-Dichlorohydrin Method. Modifications of Sobel and Werbin (*29, 30*) procedures were necessary to use the Evelyn photolectric colorimeter to measure color intensities.

APPLICATION OF SPECTROPHOTOMETRIC METHOD TO FISH OILS

Crystalline vitamin A acetate was used in these studies as the spectrophotometric, colorimetric, and biological reference standard. The well established characteristics of this form of the

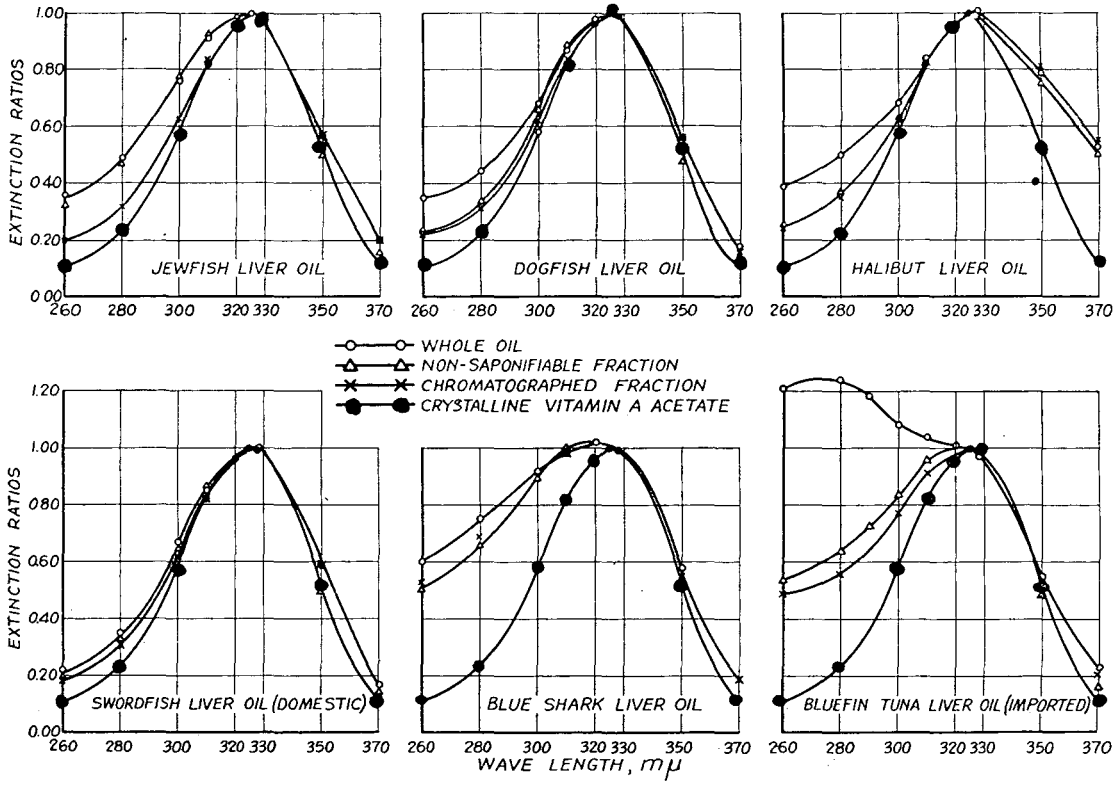

Figure 1. Absorption Characteristics of Typical Fish Liver Oils, Nonsaponifiable Fractions, and Chromatographed Residues Compared with Crystalline Vitamin A Acetate

Absorption measurements made on isopropyl alcohol solutions over range of 260 to 370 mμ by Beckman quartz spectrophotometer

Five milliliters of activated glycerol dichlorohydrin were pipetted into a standardized Evelyn colorimeter tube. One milliliter of a chloroform solution containing the vitamin A was added to the reagent, the tube was swirled to attain homogeneity and allowed to stand at room temperature, and the intensity of the resultant color was measured in a 2- to 5-minute interval after mixing the reagents. The measurements were made by the Evelyn photoelectric colorimeter equipped with a 540-mμ filter. This method of assay was standardized on the basis of crystalline vitamin A acetate and then applied to the fish oils and to their nonsaponifiable fractions.

Assay of Whole Oils by Biological Method. The U.S.P. biological method was also employed in determining the vitamin A content of the whole fish oils (*31*). The vitamin A standard was assayed at four different levels. Predetermined spectrophotometric data were used in calculating the amounts of the various oils to be fed to the groups of test animals, each of which consisted of 9 to 16 animals. The biological potencies of the various oils were evaluated from a response curve prepared from the responses made by control groups of test animals receiving different amounts of the new U.S.P. vitamin A standard (crystalline vitamin A acetate in cottonseed oil).

PRESENTATION OF DATA

The data obtained in these studies have been condensed and are presented in Tables I to VIII and Figures 1 and 2.

vitamin make possible a direct correlation between spectrophotometric and colorimetric data and biological activity.

Before proceeding toward the main objective of the present investigation, some consideration was given to the possibility of complications owing to the presence of interfering materials in the fish oils which might vitiate the results obtained, especially by the physical method of assay. According to the scientific literature, these interfering materials may absorb ultraviolet light, may react with the same reagents that react with vitamin A to form color, or may actually inhibit the formation of the color attributed to vitamin A.

Morton and Stubbs (*24*) experienced difficulty with materials present in fish oils that interfere with the quantitative measurement of vitamin A by spectrophotometric means and suggested a mathematically derived formula for use in correcting for the irrelevant absorption. However, in the experience of the authors, the Morton and Stubbs "correction" cannot be applied indiscriminately to all vitamin A carriers. In other words, there are fish oils and other vitamin A carriers which do not seem to meet the conditions imposed by the Morton and Stubbs formula. The Morton and Stubbs formula for correcting for irrelevant absorption appears to apply reasonably satisfactorily to those vitamin A carriers which show a maximum absorption at essentially the same wave length as does the vitamin A reference standard, and which also have absorption curves in the region of 300 to 350 mμ similar to that of the vitamin A reference standard. It also appears to apply to other vitamin A carriers, if first through various treatments they can be made to comply with the above conditions.

The measurement of ultraviolet light absorption before and after selective irradiation of the vitamin A carrier has also been suggested as a means of compensating for the irrelevant absorption so frequently encountered when attempting to determine the vitamin A content of some fish oils by the spectrophotometric method.

Inasmuch as the interfering materials present in vitamin A carriers may differ in their chemical constitution and in their relative occurrence, it is difficult at the present time to make accurate allowances for their effects on spectrophotometric and colorimetric measurements of vitamin A as it exists in a wide range of fish oils; hence the removal of the interfering materials is highly desirable. Saponification, chromatography, and phasic separation seem the most promising means of achieving this end. Saponification has been widely used as a means of eliminating from vitamin A carriers materials that interfere with spectrophotometric and colorimetric measurements of the vitamin; chromatography and phasic separation have been recently suggested as offering definite possibilities in this connection.

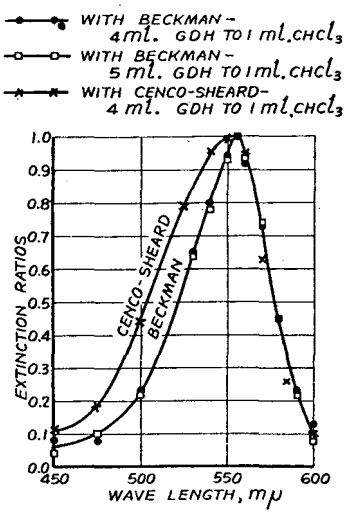

Figure 2. Absorption Characteristics of Vitamin A Acetate-Glycerol Dichlorohydrin Reaction Products

Removal of Interfering Materials by Saponification. The necessity of saponifying all fish oils before attempting to estimate their vitamin A content has been both advocated (*25*) and questioned (*23*) in recent years.

Bolomey and Sycheff (*2*), in a study of soupfin shark oils, noted that many of the substances present in the whole oils, especially in low potency oils, which interfered with the estimation of vitamin A, remain in the nonsaponifiable fraction. A somewhat similar report has been recently issued by Morton and Stubbs (*24*). In attempting to eliminate these materials through saponification, the possible loss of vitamin A must be reckoned with. Hume and Chick (*15*) are of the opinion that no loss of

Table I. Effect of Concentration of Oil in Saponification Mix on Efficiency of Vitamin A Recovery

Type of oil Saponified	Amount of Oil Saponified	Vitamin A Content of Oil Saponified[a]	Vitamin A Recovered[b]
	Grams	*U.S.P. units*	%
Swordfish liver oil (domestic)	0.25	38,050	99
	0.25[c]	38,050	101
	0.50	76,100	99
	1.00	158,200	99
	1.00[c]	158,200	101
Vitamin A ester in Wesson oil	0.25	2,110	96
	0.50	4,220	97
	1.00	8,440	93
	2.00	16,880	97
	2.50	22,100	91
	3.00	25,320	89

[a] $E^{1\%}_{1\ cm.}$ –325 mμ of whole oil × 1925.

[b] Based on ratio of $E^{1\%}_{1\ cm.}$ –325 mμ of nonsaponifiable fraction to that of whole oil.

[c] Saponified according to suggestion of Oser *et al.* (*25*).

Table II. Efficiency of Vitamin A Recovery Following Saponification

(As indicated by resaponifying nonsaponifiable fraction of swordfish oil alone and in presence of added oils)

Nonsaponifiable Residue Subjected to Resaponification[a]	Type of Oil Added	Amount of Oil Added	Total Vitamin A Saponified[a]	Vitamin A Recovered
U.S.P. units		*Gram*	*U.S.P. units*	%
15,500	None	None	15,500	99
15,500	Wesson oil	0.255	15,500	98
15,500	Wesson oil	0.258	15,500	98
15,500	Swordfish liver oil	0.249	54,900	99
15,500		0.256	56,000	99

[a] $E^{1\%}_{1\ cm.}$ –325 mμ × 1925.

Table III. Efficiency of Vitamin A Recovery

(Following saponification of a distilled vitamin A ester concentrate[a] alone and in presence of added oils)

Amount of Concentrate Used				
Weight of sample	Amount of vitamin	Type of Oil Added	Amount of Oil Added	Vitamin A Recovered
Mg.	*U.S.P. units*		*Gram*	%
Determined by spectrophotometric method				
5.0	910	None	None	99
100.0	18,200	None	None	97
4.7	860	Wesson oil	0.25	105
118.0	21,500	Wesson oil	0.25	97
4.7	860	Wesson oil	0.50	99
31.0	5,650	Wesson oil	1.00	96
64.0	11,700	Wesson oil	1.00	96
100.0	18,200	Wesson oil	1.00	93
4.7	860	U.S.P. reference	0.25	102
5.6	1,020	Cod liver oil 1	0.25	98
148.0	27,700	Cod liver oil 1	0.50	98
145.7	26,600	Cod liver oil 1	0.75	95
105.8	19,300	Cod liver oil 1	1.00	96
5.6	1,020	U.S.P. reference	0.25	99
5.6	1,020	Cod liver oil 2	0.50	97
Determined by antimony trichloride method				
5.0	910	None	None	99
60.0	10,930	None	None	99
4.6	840	Wesson oil	1.00	96
59.4	10,800	Wesson oil	1.00	98
5.0	910	Cod liver oil 1	1.00	96
5.0	910	Cod liver oil 2	0.50	96

[a] Obtained through courtesy of Distillation Products, Inc., Rochester, N. Y.

Table IV. Reproducibility of Estimates of Vitamin A Content of Fish Oils

(After oils have been chromatographed through bone meal)

Type of Fish Oil Chromatographed	Sample No.	Equivalent of Oil Chromatographed	Calcd. Vitamin A Potency of Original Oil[a]
		Mg.	*U.S.P. units*
Jewfish liver oil	1	1.00	521,000
	2	1.25	511,000
	3	2.50	508,000
	4	2.50	540,000
	5	5.00	530,000
	6	10.00	583,000
	7	10.00	593,000
	8	10.00	581,000
Bluefin tuna liver oil	9	10.00	13,650
	10	20.00	13,800
	11	25.00	13,650
	12	50.00	13,850
Halibut liver oil	13	37.50	16,800
	14	75.00	16,900

[a] $E^{1\%}_{1\ cm.}$ –325 mμ of chromatographed fraction × conversion factor (1925).

Table V. Relative Vitamin A Potency of Fish Oils and Their Nonsaponifiable Fractions and Chromatographed Residues

Type of Oil	Nonsap. / Whole Oil × 100 for $E^{1\%}_{1\text{ cm.}}$ −325 mμ			$E^{1\%}_{1\text{ cm.}}$ −325 mμ for Chromat. / $E^{1\%}_{1\text{ cm.}}$ −325 mμ × 100		Chromat. SbCl₃ / for Nonsaponifiable × 100	SbCl₃ / $E^{1\%}_{1\text{ cm.}}$ −325 mμ × 100		GDH / SbCl₃ × 100	
	$E^{1\%}_{1\text{ cm.}}$ −325 mμ	SbCl₃	GDH	For whole oil	For nonsap.		For whole oil	For nonsap.	For whole oil	For nonsap.
	%	%	%							
Jewfish liver oil[a]	100	99	104	83	84	88	96	95	89	94
Swordfish liver oil, domestic[a]	98	87	89	96	98	106	104	92	99	101
Totuava liver oil, imported[a]	99	96	104	87	89	95	92	90	92	99
Menuke liver oil, imported[a]	96	96	94	90	94	101	93	93	96	95
Swordfish liver oil, imported[a]	96	90	99	95	99	105	101	94	91	103
Soupfin shark oil[b]	96	89	102	95	99	110	97	90	93	107
Spearfish liver oil, imported[a]	95	96	101	89	93	107	87	87	91	96
Barracuda liver oil[a]	98	92	99	93	94	104	98	91	91	99
Mackerel liver oil[a]	95	94	102	93	97	105	95	93	91	98
Black cod liver oil[a]	100	94	104	97	97	102	101	95	88	97
Ling cod liver oil[a]	97	94	102	95	98	102	99	96	90	97
Albacore liver oil, 1[a]	99	95	102	92	93	99	99	94	90	97
Bonita liver oil[a]	99	97	102	96	96	100	100	97	90	96
Bluefin tuna liver oil[a]	94	88	99	86	91	101	97	90	86	97
Totuava liver oil[a]	96	89	100	92	96	111	93	86	85	95
Skip Jack liver oil, imported[a]	100	90	105	93	93	101	103	92	88	103
Skip Jack liver oil[a]	94	91	104	93	98	104	98	95	89	102
Yellow Tail liver oil[a]	97	93	99	96	99	105	98	94	91	96
Mexican shark oil[b]	94	93	101	90	95	105	92	91	94	102
Argentine shark oil[b]	96	94	104	95	100	107	95	93	96	107
Blue shark oil[b]	88	74	97	79	89	118	90	75	76	99
Halibut liver oil[a]	92	111	114	85	92	104	74	89	98	101
Sebastus Marinus liver oil[a]	95	99	106	92	98	101	92	97	94	101
Bluefin tuna liver oil, imported[a]	70	75	90	62	88	105	78	84	82	99
Albacore liver oil, 2[a]	89	89	99	87	98	104	94	95	90	100
Dogfish liver oil[b]	95	97	94	92	97	104	91	94	106	102
Cod liver, fortified[b]	96	99	101	92	96	106	88	91	95	96
U.S.P. reference cod liver oil 3	86	127	123	90	105	114	68	93	98	95

[a] Obtained through courtesy of Mead-Johnson and Company.
[b] Obtained through courtesy of Distillation Products, Inc.

vitamin A is entailed in the saponification of fish oils, as a resaponification of the nonsaponifiable matter did not result in further loss of the vitamin. These authors attributed the decrease in ultraviolet absorption following the saponification of fish oils to the removal of nonvitamin A materials which absorb ultraviolet light at 328 mμ. Jones and Haines (*16*) disagree with the general conclusion of Hume and Chick, whose results indicated that the nature of the fatty materials present in the whole oils is an important consideration. For instance, Jones and Haines found that a resaponification of the nonsaponifiable matter of a fish oil alone or in the presence of shark oil frequently resulted in a smaller decrease in the combined absorption than did resaponification in the presence of a mixture containing oleic acid, stearic acid, or halibut liver oil. But, as noted by Dann (*8*), the peroxide values of these oils and fatty acids were not known; consequently, the low vitamin values observed with the oleic and stearic acid mixtures may have been due to destruction of vitamin A by peroxides as well as to the removal of extraneous ultraviolet light–absorbing materials. On the basis of data obtained through the resaponification of the nonsaponifiable matter of five unidentified fish oils, either alone or in the presence of cottonseed oil, Oser *et al.* (*25*) reported that saponification without loss of vitamin A could be attained.

In view of the above reports, an investigation of the saponification process seemed advisable before the process was applied to a large number of fish oils. Certain facts relating to the saponification procedure appeared well established.

Vitamin A had been shown to be remarkably stable in hot alcoholic potassium hydroxide solution (*7*), and considerable variation in the method of saponification had resulted in no appreciable change in the amount of vitamin A finally extracted (*1*, *16*). Likewise, saponification and extraction of the oils under an atmosphere of nitrogen had been reported to be of no real advantage (*10*, *27*). Diethyl ether and petroleum ether were the solvents most frequently used to extract the nonsaponifiable matter from the soap solution; however, the former seemed to be the more efficient of the two (*1*, *27*, *34*). Embree (*10*), as well as others, has reported on the destructive effect of diffuse daylight on vitamin A when fish oils were dissolved in certain organic solvents. He found that the use of amber-colored glassware tends to prevent the destruction of the vitamin by light. However, Zschiele *et al.* (*35*) and Benham (*1*) reported that the diffuse light of their laboratories did not cause sufficient destruction of vitamin A to warrant the use of amber glassware during the saponification and extraction of the nonsaponifiable matter of butter and fish oils.

In view of the above reports, special attention was devoted in the present studies to the nature of the fat in the saponification mix, and to the effect of light on the vitamin A during saponification and subsequent extraction. The saponification procedure used was essentially as outlined by Wilkie (*33*).

The effect of the nature of the vitamin A carrier oil on the possible loss of the vitamin during saponification was studied from several viewpoints. In one series of experiments the concentration of the oil in the saponification mixture was varied to determine whether or not the resulting concentration of the soaps affected the amount of vitamin A eventually recovered from the saponification mixture. The data, presented in Table I, show that between the limits of 0.25 and 1.0 gram of oil, the concentration of the oil in the original sample had no effect on the efficiency of vitamin A recovery. The data obtained on two samples of oil saponified by the technique of Oser *et al.* (*25*) were essentially the same as those obtained by the Wilkie technique.

The effect of resaponification on the loss of vitamin A was also studied. In this study, the nonsaponifiable fraction of a sample of the previously mentioned swordfish oil was obtained in the usual manner, and was sampled for immediate assay and for resaponification in the media indicated in Table II. As determined spectrophotometrically, resaponification caused no significant loss of the vitamin when the resaponification was carried out under the conditions of these tests.

Finally, studies were carried out relating to the effectiveness of recovery of vitamin A added to different oils. Varying amounts of the previously mentioned distilled vitamin A ester concentrate were mixed with known amounts of three different oils, aliquots of the mixture and the unmixed components were saponified, and the vitamin A content of the nonsaponifiable fractions was determined by both the spectrophotometric and antimony trichloride techniques. From these data, the percentage recovery of the added vitamin was calculated. The results (Table III) indicated that a loss of from 1 to 4% of the vitamin A occurred during the saponification and extraction. Morgareidge (*21*) has reported that the use of isopropyl alcohol as a solvent in the spectrophotometric estimation of vitamin A results in abnormally high values for the alcohol form of the vitamin but not for the ester form. On

Table VI. Vitamin A Potency of Fish Oils, Their Nonsaponifiable Residues and of Their Respective Chromatographed Fractions as Determined by Different Methods of Assay

Type of Oil	Approx. Nonsap. Matter, %	Vitamin A Content in U.S.P. Units per Gram of Original Oil or Its Equivalent as Determined by: Biological assay of whole oil	$E^{1\%}_{1\ cm.}$ -325 mμ × 2000, for nonsap.	$E^{1\%}_{1\ cm.}$ -325 mμ × 1925 For whole oil	For nonsap.	For chromat.	$SbCl_3$ For whole oil	For nonsap.	GDH For whole oil	For nonsap.
Jewfish liver oil	55	586,200	642,000	620,000	618,000	517,000	595,000	588,000	531,000	550,000
Swordfish liver oil, domestic	13	130,500	162,200	158,200	156,000	152,800	165,000	144,100	163,000	145,800
Totuava liver oil, imported	17	106,300	149,400	146,000	144,000	127,500	134,000	129,100	123,600	128,000
Menuke liver oil, imported	17	109,300	143,400	143,800	138,000	129,300	133,800	128,000	128,000	126,000
Swordfish liver oil, imported	11	135,700	125,400	125,200	120,800	119,000	127,000	113,900	116,000	117,000
Soupfin shark oil	9	83,100	120,200	120,600	115,800	115,000	117,000	104,400	109,000	111,300
Spearfish liver oil, imported	16	75,130	102,000	103,000	98,100	91,600	89,300	85,600	81,600	82,500
Barracuda liver oil	11	42,420	85,000	83,100	81,800	77,100	81,400	74,500	74,100	73,500
Mackerel liver oil	11	75,160	83,200	84,000	80,000	77,900	79,400	74,400	71,800	73,100
Black cod liver oil	9	73,850	80,600	77,400	77,500	75,000	78,400	73,800	69,000	71,700
Ling cod liver oil	13	41,260	80,200	79,300	77,200	75,500	75,700	74,200	70,400	71,800
Albacore liver oil, 1	8	79,600	71,000	69,000	68,300	63,600	68,100	64,400	61,000	62,100
Bonita liver oil	6	57,930	62,800	60,600	60,300	58,100	60,500	58,400	54,500	55,900
Bluefin tuna liver oil, domestic	6	39,760	58,000	59,300	55,800	50,800	57,300	50,200	49,500	48,900
Totuava liver oil	9	33,420	57,200	57,400	54,900	52,500	53,500	47,400	45,300	45,100
Skip Jack liver oil, imported	14	41,170	50,200	48,500	48,300	45,000	49,800	44,600	43,800	45,900
Skip Jack liver oil	8	31,980	41,600	42,600	40,000	39,400	41,900	38,000	37,200	38,800
Yellow Tail liver oil	5	32,190	40,200	39,800	38,600	38,100	39,100	36,300	35,400	35,000
Mexican shark oil	4	24,840	33,600	34,400	32,300	30,800	31,600	29,400	29,600	30,000
Argentine shark oil	5	22,560	29,400	29,600	28,300	28,200	28,100	26,400	27,000	28,200
Blue shark oil	9	8,970	19,700	21,400	18,900	16,800	19,200	14,200	14,600	14,100
Halibut liver oil	12	9,710	19,000	19,800	18,300	16,900	14,600	16,200	14,300	16,300
Sebastus Marinus liver oil	7	19,940	17,800	18,100	17,100	16,700	16,700	16,500	15,700	16,600
Bluefin tuna liver oil, imported	5	10,520	16,100	22,200	15,500	13,700	17,300	13,000	14,200	12,800
Albacore liver oil, 2	5	12,220	15,400	16,700	14,800	14,500	15,700	14,000	14,200	14,000
Dogfish liver oil	23	10,220	8,940	9,050	8,600	8,360	8,270	8,050	8,760	8,460
Cod liver oil, fortified	24	2,410	3,820	3,850	3,680	3,540	3,390	3,340	3,200	3,220
U.S.P. reference cod liver oil 3	4	1,250	1,540	1,730	1,480	1,560	1,080	1,370	1,060	1,300

Table VII. Relation of Biological Potencies of Fish Oils to Potencies Indicated by Ultraviolet Absorption Values of Nonsaponifiable Fractions and Chromatographed Residues and by Blue Color Values of Nonsaponifiable Fractions

Type of Oil	Spectrographic Value of Nonsap. × 100 Biological Potency	Spectrographic Value of Chromat. × 100 Biological Potency	$SbCl_3$ Value of Nonsap. × 100 Biological Potency
Jewfish liver oil	105	88	100
Swordfish liver oil, domestic	120	113	110
Totuava liver oil, imported	135	120	121
Menuke liver oil, imported	126	118	117
Swordfish liver oil, imported	89	88	84
Soupfin shark oil	139	138	126
Spearfish liver oil	131	122	114
Barracuda liver oil	193	182	176
Mackerel liver oil	106	104	99
Black cod liver oil	105	102	100
Ling cod liver oil	187	183	180
Albacore liver oil, 1	86	80	81
Bonita liver oil	104	100	101
Bluefin tuna liver oil, domestic	140	128	126
Totuava liver oil	164	157	142
Skip Jack liver oil, imported	117	109	108
Skip Jack liver oil	125	123	119
Yellow Tail liver oil	120	118	113
Mexican shark oil	130	124	118
Argentine shark oil	125	125	117
Blue shark oil	211	187	158
Halibut liver oil	188	174	167
Sebastus Marinus liver oil	86	84	83
Bluefin tuna liver oil, imported	147	130	124
Albacore liver oil, 2	121	120	115
Dogfish liver oil	84	82	79
Cod liver oil, fortified	153	146	139
U.S.P. reference cod liver oil 3	118	125	110

the basis of this report, the vitamin A recovery values given in Table III may be somewhat high; however, the values agree well with those obtained by the antimony trichloride technique. Although the peroxide numbers for the Wesson oil and the two cod liver oils used, as determined by a modification of the method of Greenbank and Holm (*12*), were 2.0, 13.0, and 17.0, respectively, the expected effect of differences in peroxide numbers on vitamin recovery was not discernible.

In a study of the effect of light on the stability of vitamin A during saponification and subsequent extraction, quadruplicate samples of two fish oils were saponified and the vitamin A content of the nonsaponifiable fractions was determined by the spectrophotometric technique. Two samples of each oil were processed entirely in amber-colored glassware and two samples were processed in clear glassware. The processing was carried out in the laboratory where the ordinary window shades were employed to shield the apparatus from direct sunlight. However, no attempt was made to prevent the entrance of diffuse daylight or to dim the artificial lights. The remaining ether was removed from one sample of each set of duplicates by distillation in vacuo or by evaporation with a current of nitrogen, while the ether from the other sample was removed in the conventional manner. No difference in results could be detected.

When, under the same laboratory conditions, 22 fish oils were saponified in duplicate and the vitamin A content of their respective nonsaponifiable residues was determined by the spectrophotometric method, one duplicate being processed in amber-colored glassware and the other in clear glassware, the results did not show that the use of amber-colored glassware offered any advantage over the ordinary glassware under the conditions of these experiments. The over-all vitamin values obtained by the use of clear glassware were 99% of those obtained through the use of amber glassware, and no marked differences in vitamin preservation could be detected for any one oil. However, with other vitamin A carriers or under other experimental conditions, the use of amber-colored glassware may offer certain advantages.

From the above, it was apparent that saponification of certain vitamin A– carrying oils could be accomplished without appreciable loss of the vitamin A, whereas other oils show a slight but definite loss of vitamin A during the saponification. Hence the saponification procedure was applied to all the fish oils under investigation and the spectrographic data for the whole oils and for their nonsaponifiable residues are given in Figure 1 and Tables V, VI, VII, and VIII.

Removal of Interfering Materials by Chromatography. Recently, Glover *et al.* (*11*) reported the separation of the esters of vitamin A from the alcohol form by passing a petroleum ether solution of the vitamin through a column of defatted bone meal; the ester form was only weakly retained while the alcohol form was strongly adsorbed. Mann (*20*) had previously shown that xanthophyll from egg yolk is more tenaciously retained by this adsorbent than is β-carotene. Thus it was of interest to determine if the sterols, the oxidation products of vitamin A, or other materials present in fish oils which interfere with vitamin A determination could be separated from the vitamin A esters by chromatographing petroleum ether solutions of fish oils through bone meal.

First, in order to determine whether or not destruction of vitamin A occurred during chromatography through bone meal, four different samples of crystalline vitamin A acetate in Wesson oil were diluted with redistilled petroleum ether and passed

through columns of bone meal as previously described. Recoveries of 97, 97, 98, and 98%, respectively, of the vitamin A in the four samples were attained, as indicated by spectrophotometric measurements. The absorption curves of these eluate residues in isopropyl alcohol differed from that of crystalline vitamin A acetate in the same solvent only at wave lengths shorter than 280 mμ. In chromatographing vitamin A acetate solutions no greater amount of the vitamin was recovered when the elution was made with a mixture of 2.5% chloroform in petroleum ether, as used by Glover *et al.* (*11*), than when the elution was made with petroleum ether alone. Furthermore, the absorption curves of the former eluate residues when dissolved in isopropyl alcohol differed more markedly from the ultraviolet absorption curve of crystalline vitamin A acetate in this solvent than did those of the petroleum ether eluates.

Tests conducted with representative fish liver oils revealed that under the condition of the authors' studies a restriction of the amount of vitamin A in the aliquot chromatographed to 3000 U.S.P. or less was necessary to secure maximum reproducibility of results (see Table IV).

An examination of the absorption curves of representative fish oils, when plotted as extinction ratios *vs.* wave length (Figure 1) of the bone meal eluate residues dissolved in isopropyl alcohol, showed that they approached more closely the absorption curves for crystalline vitamin A acetate than did the corresponding curves for the whole oils. Hence, the chromatographic procedure was applied in determining the vitamin A content of each of the fish oils under investigation.

Application to Fish Oils. The results obtained through spectrophotometric examination of the isopropyl alcohol solutions of the whole fish oils and of their nonsaponifiable fractions and chromatographed residues were expressed as extinction ratios (see Table VIII), the extinction ratios were plotted against wave length, and the resulting curves were compared with a corresponding curve for crystalline vitamin A acetate. Typical data are presented in Figure 1. Likewise, the relative vitamin A potencies of various fish oils and of their nonsaponifiable fractions and chromatographed residues as determined by different methods of assay are presented in Table V, while their potencies are presented in Table VI.

From these data it is obvious that saponification and bone meal chromatography removed from the fish oils some nonvitamin A materials which absorb ultraviolet light. The nonsaponifiable matters and the chromatographed residues possessed absorption characteristics more comparable to those of pure crystalline vitamin A acetate than do those of the whole oils. On the basis of this criterion, chromatography seemed to have removed more of the interfering materials than did saponification. However, any vitamin A alcohol present in the whole oil is supposed to be retained by the bone meal and therefore would not pass into the eluate. According to Kascher and Baxter (*17*), some fish oils may contain as much as 5% of their vitamin A in the alcohol form. Thus the removal of the alcohol form of the vitamin may have contributed somewhat to the lower vitamin A values obtained through bone meal chromatography.

APPLICATION OF ANTIMONY TRICHLORIDE METHOD TO FISH OILS

The data obtained as the result of assaying the 28 fish oils and their nonsaponifiable fractions by the antimony trichloride method have been tabulated and arranged to permit comparison with corresponding data obtained by other methods of assay (see Tables V, VI, and VII). In most instances, the results obtained when this method of assay was applied to the whole oils agree well with those obtained by the spectrophotometric method. However, when the nonsaponifiable fractions of the oils were assayed by the two methods, in general the antimony trichloride method yielded lower vitamin A values than did the spectrophotometric

Table VIII. Extinction Ratios[a] of Whole Oils

(Before and after removal of interfering materials by saponification and by bone meal chromatography)

Type of Oil	Extinction Ratios for Following Wave Lengths																					
	At 260 mμ			At 280 mμ			At 300 mμ			At 310 mμ			At 328 mλ			At 350 mμ			At 370 mμ		
	W.O.	N.S.	C.R.	W.O.	N.S.	C.R.	W.O.	N.S.	C.R.	W.O.	N.S.	C.R.	W.O.	N.S.	C.R.	W.O.	N.S.	C.R.	W.O	N.S.	C.R.
Jewfish liver oil	0.36	0.33	0.20	0.49	0.48	0.32	0.77	0.79	0.63	0.92	0.93	0.83	0.99	0.97	0.99	0.57	0.50	0.57	0.20	0.16	0.19
Swordfish liver oil, domestic	0.22	0.20	0.19	0.35	0.33	0.32	0.66	0.65	0.62	0.84	0.87	0.83	1.00	0.99	1.00	0.59	0.51	0.59	0.21	0.16	0.21
Totuava liver oil, imported	0.23	0.27	0.19	0.39	0.40	0.32	0.68	0.69	0.63	0.86	0.88	0.85	0.99	0.97	1.01	0.62	0.54	0.64	0.26	0.21	0.25
Menuke liver oil, imported	0.27	0.24	0.22	0.39	0.36	0.34	0.66	0.67	0.64	0.85	0.88	0.84	1.00	0.99	1.00	0.62	0.55	0.62	0.25	0.20	0.23
Swordfish liver oil, imported	0.21	0.19	0.16	0.34	0.32	0.25	0.64	0.64	0.59	0.84	0.86	0.81	1.00	0.99	1.00	0.60	0.52	0.60	0.22	0.17	0.22
Soupfin shark oil	0.25	0.22	0.19	0.38	0.36	0.33	0.67	0.68	0.64	0.86	0.89	0.85	0.99	0.98	1.00	0.55	0.47	0.56	...	...	0.18
Spearfish liver oil, imported	0.34	0.31	0.26	0.45	0.43	0.39	0.70	0.70	0.67	0.86	0.89	0.85	1.00	0.99	1.00	0.63	0.58	0.63	0.29	0.26	0.29
Barracuda liver oil	0.25	0.21	0.20	0.38	0.35	0.34	0.63	0.62	0.61	0.83	0.84	0.81	1.00	0.99	1.01	0.68	0.59	0.67	0.32	0.25	0.31
Mackerel liver oil	0.25	0.21	0.19	0.37	0.33	0.32	0.65	0.64	0.61	0.84	0.86	0.82	1.00	0.99	1.00	0.61	0.53	0.59	0.23	0.17	0.21
Black cod liver oil	0.21	0.18	...	0.34	0.30	...	0.62	0.63	...	0.82	0.85	...	1.01	0.99	0.99	0.64	0.56	0.59	0.27	0.22	0.23
Ling cod liver oil	0.21	0.17	0.17	0.33	0.31	0.30	0.61	0.62	0.59	0.81	0.85	0.81	1.00	0.99	1.00	0.62	0.55	0.61	0.24	0.20	0.24
Albacore liver oil	0.32	0.19	0.16	0.36	0.31	0.29	0.64	0.63	0.60	0.84	0.86	0.81	1.00	0.99	1.00	0.60	0.51	0.59	0.21	0.15	0.20
Albacore liver oil, 1	0.22	0.18	0.19	0.34	0.32	0.32	0.62	0.62	0.60	0.82	0.85	0.82	1.00	0.99	1.00	0.62	0.54	0.61	0.25	0.20	0.23
Bonita liver oil	0.37	0.30	0.23	0.50	0.44	0.37	0.73	0.72	0.65	0.88	0.91	0.84	0.99	0.99	0.99	0.58	0.51	0.59	0.22	0.17	0.21
Bluefin tuna liver oil, domestic	0.19	0.33	0.29	0.39	0.46	0.43	0.70	0.73	0.68	0.87	0.89	0.85	1.00	0.99	1.00	0.68	0.60	0.66	0.38	0.32	0.35
Totuava liver oil, domestic	0.42	0.37	0.34	0.49	0.44	0.41	0.67	0.65	0.62	0.84	0.85	0.81	1.00	0.98	1.00	0.63	0.55	0.61	0.25	0.19	0.23
Skip Jack liver oil, imported	0.35	0.27	0.21	0.45	0.37	0.30	0.66	0.65	0.51	0.83	0.85	0.82	1.00	0.99	1.00	0.61	0.53	0.51	0.25	0.19	0.20
Skip Jack liver oil	0.24	0.21	0.20	0.38	0.34	0.34	0.62	0.61	0.59	0.82	0.84	0.80	1.01	1.00	1.02	0.71	0.64	0.73	0.37	0.30	0.35
Yellow Tail liver oil	0.37	0.29	0.27	0.50	0.42	0.41	0.75	0.73	0.69	0.90	0.91	0.87	0.99	...	0.99	0.64	0.47	0.54	...	...	0.16
Mexican shark oil	0.30	0.26	0.23	0.43	0.39	0.36	0.71	0.71	0.67	0.89	0.91	0.87	0.99	...	0.99	0.64	0.46	0.54	...	...	0.16
Argentine shark oil	0.60	0.51	0.53	0.75	0.67	0.69	0.92	0.91	0.90	0.99	1.00	0.99	1.00	0.97	0.98	0.58	0.52	0.55	...	...	0.24
Blue shark oil	0.39	0.26	0.24	0.50	0.37	0.36	0.68	0.63	0.62	0.83	0.83	0.81	1.01	1.00	1.01	0.79	0.75	0.81	...	...	0.54
Halibut liver oil	0.26	0.17	0.18	0.37	0.27	0.28	0.63	0.60	0.60	0.82	0.83	0.83	1.00	1.00	1.00	0.61	0.53	0.63	0.53	0.51	0.25
Sebastus Marinus liver oil	1.21	0.47	0.47	1.24	0.64	0.59	1.08	0.84	0.77	1.04	0.96	0.91	0.97	0.97	0.98	0.55	0.49	0.53	0.24	0.19	0.17
Bluefin tuna liver oil, imported	0.40	0.32	0.29	0.50	0.41	0.39	0.68	0.65	0.61	0.85	0.86	0.82	1.00	0.99	1.01	0.63	0.55	0.63	0.28	0.22	0.26
Albacore liver oil, 2	0.35	0.23	0.22	0.44	0.34	0.32	0.69	0.66	0.62	0.86	0.88	0.83	0.99	0.99	0.99	0.56	0.48	0.56	...	...	0.17
Dogfish liver oil	0.44	0.29	0.29	0.49	0.39	0.39	0.69	0.69	0.64	0.88	0.88	0.84	0.99	0.98	0.99	0.56	0.47	0.56	...	...	0.18
Cod liver oil, fortified	0.92	0.27	0.49	0.88	0.38	0.58	0.82	0.65	0.70	0.91	0.85	0.86	0.99	0.99	0.99	0.59	0.53	0.60	0.24	0.18	0.20
U.S.P. reference cod liver oil 3																					

[a] Ratio of optical density at specified wave length compared to optical density at 325 mμ. W.O. whole oil, N.S. nonsaponifiable fraction, and C.R. chromatographed residue.

method; the vitamin A potencies determined by the antimony trichloride method were 92 ± 3% of those obtained by the spectrophotometric method for 21 of the 28 oils examined. In fact, the potencies indicated by applying the antimony trichloride method to the nonsaponifiable fractions were more comparable to those found when the chromatographed residues were examined by the spectrophotometric method of assay or those obtained by assaying the nonsaponifiable fractions by the glycerol dichlorohydrin method.

APPLICATION OF GLYCEROL 1,3-DICHLOROHYDRIN METHOD TO FISH OILS

The reaction of glycerol 1,3-dichlorohydrin with vitamin A to form a colored product had been recommended (*29*, *30*) as the basis of a method for the estimation of vitamin A, and seemed to merit further study. In the previous studies of this method of assay, the Beckman quartz spectrophotometer and the Coleman spectrophotometer had been used as means of measuring the amount of color formed. Inasmuch as it was desirable to compare the results obtained by this method of assay with those obtained by the antimony trichloride method, it seemed advisable to use the same instrument in the two methods of assay.

As the Evelyn photoelectric colorimeter has been widely and satisfactorily used in connection with the antimony trichloride method (*4*), it seemed to be the logical instrument for use in the present studies. However, certain modifications of the original procedure were found necessary (*29*, *30*) especially with respect to the amount of reagent used, because the macro system of the Evelyn requires a minimum of 6 ml. of solution. To provide for this minimum volume, the ratio of glycerol dichlorohydrin reagent to vitamin A solution was changed from 4 ml.:1 ml. to 5 ml.:1 ml., respectively. But before doing this, some tests were made for the purpose of determining whether this change in the ratio of reagent to vitamin solution affected the outcome of the assay.

Absorption measurements of the colored solutions resulting from the two ratios of samples and reagents were made by means of the Beckman spectrophotometer, using the vitamin A acetate standard. Similar measurements were made with a Cenco-Sheard spectrophotelometer. Absorption curves prepared from the data obtained with the Beckman spectrophotometer (Figure 2) show that essentially the same color is produced by the two ratios of reagents. However, an examination of the light absorption data obtained by means of the Cenco-Sheard spectrophotelometer indicated a somewhat broader absorption curve with a maximum at 550 mμ instead of at 553 mμ. Color measurements made with the latter instrument approximate more closely those made with the Evelyn colorimeter, as the characteristics of the incident light were nearly comparable. Sobel and Werbin (*29*) have reported somewhat similar instrumental differences from their studies of the glycerol dichlorohydrin reaction.

In continuing these studies, the $L_{1\text{ cm.}}^{1\%}$–540 mμ values of the colors produced by adding either 4 or 5 ml. of the glycerol dichlorohydrin reagent to 1-ml. aliquots of a series of chloroform solutions of vitamin A acetate were determined by means of the Evelyn photoelectric colorimeter. This was accomplished by using the micro and macro system of the Evelyn to measure the colors produced, respectively, by the 4 to 1 and the 5 to 1 ratio of solutions to reagents. For this purpose, a 540 mμ filter was used, because it is usually supplied with the instrument, and the 540-mμ maximum of the transmitted light is not far displaced from the maxima of the color under consideration. The mean $L_{1\text{ cm.}}^{1\%}$–540 mμ values and the average deviation from the mean were found to be, for the 4 to 1 and for the 5 to 1 reagent ratio systems, respectively, 943 ± 15 and 949 ± 13. With the Beckman spectrophotometer, a $E_{1\text{ cm.}}^{1\%}$–553 mμ value of 1108 was obtained on the same solutions when a reagent-sample ratio of 4 to 1 was used. Thus, the data again suggest that the essential characteristics of the color produced are the same for the two ratios of solutions to reagents.

Finally, a linear relationship between optical density and concentration of vitamin A was observed between the limits of 1.7 and 13.7 micrograms of vitamin A acetate per ml. of chloroform solution when the intensities of the color were determined on either the macro or micro systems of the Evelyn photoelectric colorimeter in the manner previously described.

The color produced by the reaction of the activated glycerol 1,3-dichlorohydrin with vitamin A has been reported (*29*, *30*) to remain stable for at least 8 minutes after development of maximum intensity. In this laboratory, however, the maximum intensity of the colors produced by the reaction of either the authors' own activated reagent, or the commercial activated reagent with vitamin A acetate, developed approximately 2 minutes after the mixing of the reagents with the sample, remained essentially stable for 3 additional minutes, and then declined in intensity. The stability of the color did not appear to be influenced by the two ratios or reagents to samples nor by the type of instrument used in making the color measurements. Sobel and Werbin (*29*, *30*) suggested the use of a constant temperature bath of 25° C. as a means of bringing about uniform color development. However, a brief study in this laboratory indicated that, within the temperature range of 18° to 36° C., temperature has no appreciable effect on the intensity of the colors produced.

The results obtained by the glycerol dichlorohydrin method showed excellent agreement with those obtained by the antimony trichloride method when applied to the nonsaponifiable fractions of the fish oils. However, when the two colorimetric methods were applied to the whole oils, the glycerol 1,3-dichlorohydrin method yielded lower vitamin A values for the majority of the oils. This discrepancy perhaps may be ascribed to a greater degree of inhibition of color formation with the glycerol 1,3-dichlorohydrin reagent than with the antimony trichloride reagent, owing to the presence of extraneous materials in the whole fish oils Sobel and Werbin (*28*) noted a favorable correlation similar to the above when the two methods of assay were applied to the nonsaponifiable fractions of fish oils. However, when the two methods of assay were applied to the whole oils, these authors noted good agreement between the results obtained, except for several oils which they described as being "atypical" because of abnormal color formation with the reagent. For these latter oils, the vitamin A values obtained by the glycerol 1,3-dichlorohydrin method of assay were much lower than those obtained by the antimony trichloride method.

BIOLOGICAL METHOD OF ASSAY

The results of the biological assays are presented in Tables VI and VII. Because these assays were carried out in accordance with the U.S.P. technique and a detailed discussion of the results is to be published, an extended discussion seems unnecessary in this connection. Instead, the data have been compiled and tabulated to permit a ready comparison between the results obtained by this method of assay and those obtained for the same fish oils by other methods of assay. With the majority of the fish oils examined (19 out of 28) the vitamin A potency determined by the biological assay of the whole oil was lower than that indicated by any other method of assay.

SUMMARY

The vitamin A content of 28 fish oils, representative of fish oils of commerce, was determined by the spectrophotometric, antimony trichloride, glycerol 1,3-dichlorohydrin, and biological methods of assay. The vitamin A content of the nonsaponifiable fraction of these oils was determined by the spectrophotometric, antimony trichloride, and glycerol dichlorohydrin methods of assay. In addition, the chromatographed eluate of each of the 28 fish oils was assayed by the spectrophotometric procedure.

The resulting data show that much of the extraneous material normally present in some fish oils, which interferes with vitamin A determination by chemico-physical methods, may be eliminated by saponification and by chromatography without serious loss of vitamin. As a whole, the maximum vitamin A potency was indicated by the spectrophotometric method of assay when applied to the whole oils, whereas the minimum potency was indicated by the biological method of assay. The antimony trichloride and glycerol dichlorohydrin methods of assay, when applied to the nonsaponifiable fractions, yielded data somewhat more comparable to those obtained by the biological assay than were the data obtained by the spectrophotometric procedure when conversion factors of 2000 and 1925 were used in the calculations.

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Determination of Particle Morphology from Quantitative Analyses

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A system of analyses is given for the determination of morphological features of powder particles. The method is applicable to particles composed of two or more phases distributed in such a way as to be characteristic of the particle structure. Deductions about particle morphology are made from the manner in which quantitative analyses for a chosen component vary with particle size. As an example, a commercial ammonia synthesis catalyst is analyzed by this method and found to have a mosaiclike microstructure. The small particles tend to be single-celled, bearing a potassium-silicon deposit primarily on the outer surface; the larger particles tend to consist of more than one cell, and contain the deposit along the cell boundaries as well as on the external surface.

IN APPLICATIONS employing powdered materials for special purposes, the form and physical structure of the particles are usually of considerable importance. This is especially true in the case of certain powdered catalysts used in petroleum refining processes, where the quality of these catalysts is found to depend on physical as well as chemical characteristics.

If several phases are present in the particles, morphological investigations may become rather difficult; in addition to its physical form, a particle may have a characteristic structure due to a systematic distribution of the component phases. It is in just such cases that the method described here is particularly valuable in supplying information not readily available by other analytical procedures.

Using the proposed method, the morphology of the particles is deduced from the manner in which quantitative analyses for selected components vary with particle size. Some desirable features of this method are:

Results are in terms of average morphology, because in each quantitative analysis a large number of particles are included.

The distribution of minor constituents can be ascertained

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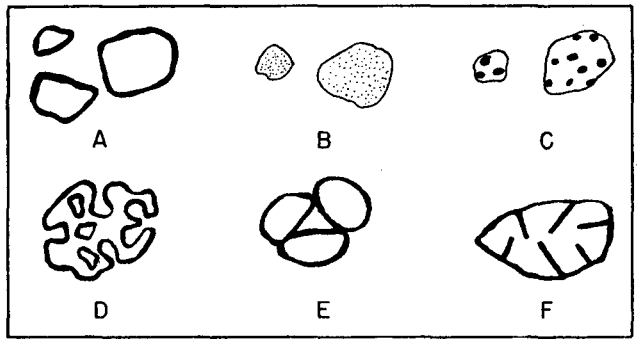

Figure 1. Particles That Can Be Investigated by Method of Morphological Analysis Based on Quantitative Analyses

A have a deposit on external surfaces; *B* and *C* represent distribution of deposit throughout particles; *D* contains a deposit internally as well as on its external surface; *E* represents an agglomerate built up from particles of type *A*; *F* represents a "pseudo agglomerate"

The deduced morphology is in terms of the constituent phases, thus facilitating correlation of particle morphology with particle behavior.

In the following sections, a reference phase is considered as a deposit present in the catalyst particles. Some of the more commonly occurring simple cases are treated mathematically, and an illustration of the application of the method is given.

DEPOSIT ON EXTERNAL SURFACE

Consider a sample consisting of solid particles with a surface deposit, which need not be of uniform thickness but has an average thickness, t, for particles of all sizes. The exact shape of the particles need not be too rigidly specified, other than that they should not be excessively elongated, have deep fissures, etc. The type of particles under consideration is illustrated in Figure 1*A*. Denoting the diameter of such a particle by D, the volume then can be expressed as CD^3 where C is an appropriate constant of proportionality.

The volume of the deposit is the total volume of the particle minus the volume of the interior portion containing no deposit. Hence, W, the weight per cent of the deposit, is

$$W = \frac{\{CD^3 - C(D-2t)^3\}\rho}{C(D-2t)^3\rho_0 + \{CD^3 - C(D-2t)^3\}\rho} \times 100$$

$$= \frac{\frac{\rho}{\rho_0}\left\{\frac{6t}{D} - \frac{12t^2}{D^2} + \frac{8t^3}{D^3}\right\}}{\left(1-\frac{2t}{D}\right)^3 + \frac{\rho}{\rho_0}\left\{\frac{6t}{D} - \frac{12t^2}{D^2} + \frac{8t^3}{D^3}\right\}} \times 100 \qquad (1)$$

where ρ is the density of the deposit and ρ_0 is the density of the rest of the particle.

If the thickness of the deposit is very small compared with the particle diameter, Equation 1 can be simplified to

$$W = \frac{6t\rho}{\rho_0 D} \times 100 \qquad (2)$$

Using log-log coordinates, the curves assume easily recognizable characteristic shapes. For instance, Equation 2 in the logarithmic form becomes

$$\log W = -\log D + \log \frac{6t\rho}{\rho_0} + 2 \qquad (3)$$

which is a straight line of slope -1 (see Figure 2*G*). If Equation 1 is plotted on these coordinates, the shapes of the curves for different values of t will all be alike; the curves will be merely displaced with respect to each other in the log D direction. Ratio ρ/ρ_0, however, does have some influence on the shapes of the curves. This is illustrated in Figure 2, *A*, *B*, and *C*, for which ρ/ρ_0 has the values 10, 1, and 0.1, respectively, and the value of t is 5 units. A particle smaller than $2t$ will be regarded as having no core, but as being composed entirely of the surface-layer material. In general, each curve can be seen to have a horizontal portion at $W = 100$ for $D \leq 2t$, a portion having a slope approaching -1 corresponding to Equation 2, and a transition section joining these two extremes. This intermediate section is steeper for smaller values of density of the surface material—i.e., smaller values of ρ/ρ_0. The linear portions of the curves having slopes of -1 may extend up to rather large values of W, as in the case of curve *B*, for example. The approximate relationship for a surface deposit, Equation 2, can be said to be useful up to about 20 (or more) weight % of the deposit, depending on the value of ρ/ρ_0.

From an experimental curve of log W versus log D, one can easily determine whether a surface deposit is present. (This conclusion is highly probable but not unique. It is possible to imagine some freak distributions that will give identical log W–log D curves. Such possibilities can generally be ruled out from other available information about the powder particles.) Furthermore, if the particles are of type *A*, Figure 1, a complete curve can yield the following additional information:

1. The departure of the curve from the horizontal occurs at $D = 2t$; thus the approximate value of t, the average thickness of surface layer, can be obtained.
2. From the straight portion of the curve corresponding to Equation 3 (for large values of D) the value of

$$t\rho/\rho_0 = \frac{1}{6}\frac{WD}{100}$$

can be determined.

3. From the steepness of the intermediate portion of the curve, the value of ρ/ρ_0 can be estimated. Because ρ_0 usually is known or can be determined, one can in theory obtain values for t and ρ from the relationships just mentioned. In practice some departures from idealized conditions are frequently encountered; these can often be interpreted to yield additional useful information.

UNIFORMLY DISTRIBUTED DEPOSIT

If a deposit is uniformly distributed throughout the particles, as indicated in Figure 1*B*, the weight per cent of the deposit will be independent of particle size (*H*, Figure 2). On a microscopic scale, the deposit need not be very finely dispersed but may be rather coarsely dispersed, as indicated in Figure 1*C*.

DEPOSIT ON EXTERNAL SURFACE AND IN INTERIOR OF PARTICLE

A variety of particle types can be found for which a deposit is found not only on the externally exposed surface but also in the interior of the particles. An illustration of a particle of this type is given in Figure 1*D*.

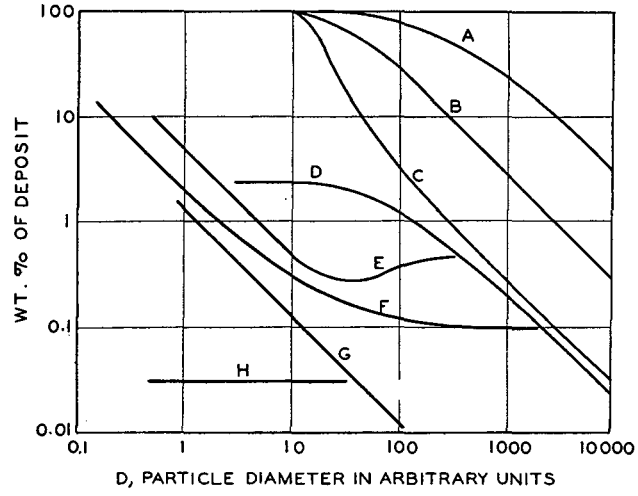

Figure 2. Characteristic Curves for Several Types of Particle Morphology

A, *B*, and *C* are for a surface deposit in which the ratios of the density of surface deposit to base material are 10, 1, and 0.1, respectively. *D* is for a deposit whose concentration decreases exponentially with depth below the surface. *E* is for a sample in which the large particles tend to be agglomerates and the small particles tend to be single; the deposit is in the form of a surface layer. *F* is for particles having a uniform internal distribution of a deposit in addition to a surface layer. *G* is for a thin surface deposit and *H* is for a uniform deposit throughout the particles

Let the total deposit, W, be written:

$$W = W_1 + W_2 \tag{4}$$

where W_1 is the external deposit and W_2 the internal deposit. For simplicity, let W_1 be given by Equation 2 and let W_2 be independent of particle size. For convenience quantities Q and K are defined as follows:

$$K = 6t\rho/\rho_0 \tag{5a}$$

$$Q = W_2/100K \tag{5b}$$

Then

$$W = \{K/D + KQ\} \times 100$$

$$\log W = \log \left(\frac{1}{D} + Q\right) + \log K + 2 \tag{6}$$

The curve given by Equation 6 is plotted in Figure 2*F*. It can be shown that the values of Q and K do not affect the shape of the curve; they merely determine the amount by which the curve is translated from an arbitrary origin. This fact makes it possible readily to evaluate Q and K by comparing a given curve with a curve for arbitrarily chosen values Q_0 and K_0:

$$\log W = \log \left(\frac{1}{D} + Q_0\right) + \log K_0 + 2 \tag{7}$$

Parameters Q and K can then be written in terms of Q_0 and K_0 as follows:

$$Q = qQ_0 \tag{8a}$$

$$K = kK_0 \tag{8b}$$

where q and k are constants to be determined. Equation 6 can then be rewritten

$$\log W = \log \left(\frac{1}{qD} + Q_0\right) + \log K_0 + \log qk + 2 \tag{9}$$

The displacement of curve 9 with respect to 7 can be expressed in terms of translations in the two coordinate directions by applying well-known methods of analytical geometry to this problem. The required results are:

$$\text{Translation in the log } D \text{ direction} = -\log q \tag{10a}$$

$$\text{Translation in the log } W \text{ direction} = \log qk \tag{10b}$$

The values of q and k are thus obtained, which lead to the evaluation of Q and K by means of Equations 8a and 8b.

The ratio of internal deposit to external deposit for any particle size is then simply

$$\frac{W_2}{W_1} = QD \tag{11}$$

Furthermore, the average thickness of the external deposit can be estimated from the value of K in conjunction with Equation 5a.

If the particles are known to be porous, an effective pore diameter can be obtained.

Let the simplifying assumptions be made that the particles and the pores are spherical. If the fraction, F, of a particle is occupied by pores, the average number of pores in a particle is

$$\frac{\text{Volume occupied by pores}}{\text{volume of one pore}} = \frac{FD^3}{d^3} \tag{12}$$

where d is the pore diameter. The weight per cent of material deposited on the pore wall is then

$$W_2 = \frac{(\text{number of pores per particle}) \times (\text{surface area of one pore})}{(\text{volume of one particle}) \times (1 - F) \times \rho_0} \times t\rho \times 100$$

$$= \frac{6Ft}{d(1 - F)} \frac{\rho}{\rho_0} \times 100 \tag{13}$$

If t and F are known from other measurement, Equation 13 can be solved for the effective pore diameter, d.

OTHER DISTRIBUTIONS

Although the mathematical development could be greatly extended to many more cases, it is felt that the method has been sufficiently well indicated, and that special cases can be handled as they arise.

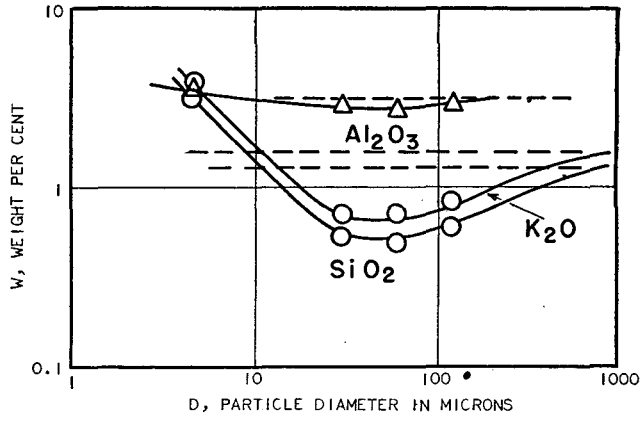

Figure 3. Experimental Curves for Commercial Ammonia Synthesis Catalyst

Dotted lines are values obtained for over-all sample

It may be of interest to note briefly the other curves in Figure 2. D has been worked out for the case of a contaminant diffusing into the particles, the concentration of the contaminant decreasing exponentially with depth below the surface. E is for a sample in which particles smaller than a certain size bear a surface deposit, and larger particles are, in addition, agglomerate in nature, as illustrated in Figure 1*E*. The curve is characterized by a straight-line portion having a slope of -1 for the small particle size range, and by an asymptotic approach to a constant value corresponding to the over-all sample, as the particle size becomes very large. In the transition region between these extremes, the curve may go through a minimum as shown in Figure 2*E*; in the particle size range indicated, the tendency for the formation of the dip in the curve is favored by an increasing proportion of single particles in the mixture, and the presence of an increasing portion of larger-than-average elementary particles in the agglomerates. Particles having the structure sketched in Figure 1*F*, will also give rise to the same type of curve.

Application. As an illustration of the type of information that can be educed from data on quantitative analyses, this method of morphological analysis may be applied to a sample of commercial catalyst which was produced for synthesis of ammonia.

The material is primarily ferriferrous oxide (Fe_3O_4), containing potassium, silicon, aluminum, and several other elements as minor components. The presence of these minor constituents, in conjunction with the method of preparation, has a pronounced effect on determining the microstructure of the catalyst material. In the preparation of the catalyst, a solid mass is produced by a fusion process, and particles between No. 2 and No. 8 standard sieve sizes are obtained by crushing. This rather coarse commercial product was ground in an all-steel laboratory ball mill to produce a powder that would pass through a No. 80 standard sieve. A rather wide particle size distribution was thus obtained, the particles ranging from the order of a micron to nominally 177 microns in diameter. A separation of the particles into size fractions was effected by air elutriation, using the Roller (*2*) technique. Four cuts were obtained: 0 to 20, 20 to 40, 40 to 80, and 80 to 177 microns. For the last three cuts the average particle sizes were taken to be approximately 30, 60, and 125 microns, respectively; whereas for the fines, an average particle size of 3.8 microns was determined statistically with the aid of a measuring microscope.

Quantitative analyses were made for potassium, silicon, and aluminum on the various particle size fractions. The results, in terms of potassium oxide, silica, and alumina, are shown plotted against average particle size on log-log coordinates in Figure 3. The dotted lines, indicating the values for very large particle size, are the values that were obtained for the composite catalyst before a particle size separation was made.

The K_2O curve has the same shape as the SiO_2 curve, and the curves are displaced vertically with respect to each other. This implies that the concentration of potassium is proportional to the

silicon concentration in each cut, and that potassium is localized in the same regions on the particles as is the silicon. The SiO_2 and K_2O curves resemble curve *E* in Figure 2, going through a minimum and having a slope of −1 for small particle sizes. *E* was constructed for the case in which the small particles have only a surface deposit and the larger particles, in addition, tend to be agglomerated as indicated in Figure 1, *E* and *F*. On the basis of sample preparation, particles of type *E* can be ruled out in favor of type *F* particles. The analysis so far leads to the conclusion that the silicon and potassium compounds are not very soluble in the Fe_3O_4 matrix but are probably homogeneously mixed with each other in a slag which is found on the surface of small particles and also as inclusions in the larger particles.

Independent investigations on the solubility of the potassium in this solid system have not been carried out to the writer's knowledge. However, there have been some experiments on the solubility of special potassium compounds in Fe_3O_4.

Michel's (*1*) experiments in measuring magnetic properties of various solid solutions indicated that potassium ferrite is soluble to a limited extent in Fe_3O_4. The solubility of potassium aluminate in Fe_3O_4 was studied by Wyckoff and Crittenden (*3*), who used the expansion of the crystal lattice constant of Fe_3O_4 as a criterion of solid solution. No expansion was detected, so their results were inconclusive. If the potassium were uniformly dispersed in the Fe_3O_4, the curve of weight per cent versus particle size would be horizontal. That this is not the case is very strong indication that the potassium compound present is not soluble to any great extent in Fe_3O_4, and lends some support to the hypothesis that a potassia-silica glass may be formed. This amorphous material would hardly be expected to go into solid solution in the magnetite.

100 Microns

Figure 4. Photomicrograph of Ammonia Synthesis Catalyst Showing Mosaiclike Structure

On the basis that the slag containing silicon and potassium exists as inclusions in the massive magnetite before grinding, it might be asked whether the rise in K_2O and SiO_2 curves for small particle size might not be due to the preferential grinding of the presumably softer slag. Although such a process, producing fine slag particles, will enrich the small-particle fraction in silicon and potassium, there is nothing in this mechanism to predict a slope of −1 for the curve at small *D*-values, although such a slope can occur as a special case. It is possible also to account for the dip in the curve by postulating rather large slag inclusions, such that upon crushing, some of the particles will consist largely of slag, whereas others will contain very little slag. Preferential grinding of the slag particles will impoverish the intermediate particle size range of slag material, thus causing a minimum in the curve.

Although this alternative structure is a possibility, it does not fit in very well with the microscopic examination of the catalyst material depicted in Figure 4. On the other hand, this photomicrograph does support the concept of a mosaiclike particle structure. However, the limited view in the photograph is not adequate for determining average values of cell dimensions. Preferential grinding of slag material probably does occur to a limited extent, but the data are not sufficiently refined or extensive to show this with certainty. Whether or not the process occurs can be determined by subjecting several samples of the catalyst to various degrees of severity of grinding, and constructing a set of curves similar to those in Figure 3. A deepening of the dip and other changes in the curve characteristics can be expected with increasing severity of grinding.

Returning to the examination of the data presented in Figure 3, it will be observed that the alumina curve is almost horizontal, indicating that the bulk of the alumina is rather uniformly dispersed in the Fe_3O_4. Similar conclusions regarding the uniformity of distribution of the aluminum atoms were also obtained by Wyckoff and Crittenden (*3*) from their studies on ammonia synthesis catalyst. They explain this by the mechanism of alumina combining with ferrous oxide, present in the catalyst to a small extent, to form ferrous aluminate, which they found enters into solid solution with Fe_3O_4. As γ-alumina, ferrous aluminate ($FeO.Al_2O_3$), and Fe_3O_4 all have the spinel type structure and their lattice parameters are of the same order of magnitude, a solid solution of γ-alumina or ferrous aluminate in Fe_3O_4 might be reasonably suspected. Returning to Figure 3, the Al_2O_3 curve departs a little from the horizontal, and shows a slight tendency to follow the K_2O and SiO_2 curves. This suggests that the concentration of aluminum in the slag is slightly higher than in the magnetite.

The bits of information can now be fitted together to give a rather informative picture of the microstructure of ammonia synthesis catalyst, related to the method of catalyst preparation. As the molten mass of catalyst material cooled, the potassium and silicon compounds present, being insoluble in the matrix, separated out as slag inclusions in a characteristic manner to outline irregular (and possibly incomplete) three-dimensional mosaic cells. The aluminum atoms, however, are rather uniformly dispersed in the catalyst material. The slag inclusions constitute weak points structurally, so that upon grinding, the catalyst breaks up along the mosaic cell boundaries demarcated by the slag inclusions. On the average, particles smaller than about 30 microns tend to be single-celled, whereas particles greater than 30 microns tend to be multicelled. From Figure 3 it can be seen, however, that the transition is not sharp. The small particles have potassium and silicon only on their external surfaces; however, the deposit is probably not evenly distributed. The large particles contain deposits not only on their external surfaces but also internally along the mosaic cell boundaries.

CONCLUSIONS

Although further potentialities of the method could be demonstrated by additional illustrations, they could to a large extent be inferred from the general development. The variety of information obtainable from this type of morphological analysis include deductions about:

1. General particle morphology, such as whether the particles are solid or porous, single or agglomerate, etc.
2. Distribution of deposits in and on the particles
3. Estimate of surface layer thickness
4. Influence of the methods of preparation of physical characteristics of the particles
5. Surface area and pore size for coarsely porous particles
6. Progressive changes in particle characteristics from several analyses made in chronological order

The maximum usefulness of this method is in combination with other methods of analysis, other physical and chemical facts, and the general history of the particles. An analysis of this sort that is consistent with all the available facts not only can give a reliable description of particle morphology, but can also

be helpful in correlating and integrating these facts into a coherent body of useful information.

ACKNOWLEDGMENT

The writer takes pleasure in expressing his appreciation to R. N. Watts for supplying the ammonia synthesis catalyst in various particle size ranges, F. M. Long for chemical analyses on these samples, J. S. McIlhenny for particle size determinations, C. F. Gray for the photomicrograph of the unground catalyst, and G. H. Walden for many helpful suggestions.

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RECEIVED November 22, 1948

Determination of Water in Hydrogen Chloride

By Means of Karl Fischer Reagent

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A rapid titrimetric procedure employing the Karl Fischer reagent has been developed for the determination of small amounts of water in gaseous hydrogen chloride. The standard deviation in the results obtained with this method is 0.5 mg. of water for samples of known composition. Hydrocarbon gases in the C_4 range, which may be present in the hydrogen chloride of modern petroleum refinery operations, do not interfere; mercaptans (thiols) and hydrogen sulfide, which are oxidized by the reagent, constitute a source of error, but a correction may be applied if the amount of each is known.

THE utility of the Fischer reagent, originally used for the determination of water in sulfur dioxide–hydrocarbon mixtures (*3*), has since been extended to the determination of water in various substances and of water liberated in certain reactions. Such applications include the determination of water in practically all kinds of materials. It has also been used successfully in determining water in liquid sulfur dioxide (*2*), alkylation sulfuric acid (*4*), and hydrogen fluoride (*7*).

Inasmuch as hydrogen chloride is acidic and gaseous in nature, a pyridine-methanol solvent is employed in the collection of the sample; pyridine serves to bind the gas and methanol to dissolve the resulting pyridinium hydrochloride. Sufficient pyridine must be present to bind all the hydrogen chloride introduced, for in the presence of uncombined hydrogen chloride the action of Fischer reagent is unreliable.

In his original work, Fischer titrated the samples directly with the reagent and used as the end point the first permanent appearance of a brown iodine color. Although this direct titration is satisfactory, the end point is rather difficult to detect, so several other titration procedures have been tried. Back-titration of an excess of reagent with a water-in-methanol solution has been employed by several investigators who used potentiometric (*1*), dead-stop (*8*), and cathode ray magic-eye (*5*) devices to detect the end point. However, excellent results can be obtained by a simple back-titration to an end point detected visually as a color change from red-brown through gold-brown to a final brassy yellow.

REAGENTS

Water in Methanol. Because this solution is not standardized, it is prepared simply by adding approximately 5 ml. of water to 2 liters of C.P. methanol.

Sample Solvent. The solvent consists of 4 parts by volume of dry pyridine and 3.5 parts of redistilled methanol.

Fischer Reagent. Gaseous sulfur dioxide, led through a drying tube containing Drierite, is passed into 226 grams of pyridine in a small bottle until 55 grams have been added. The pyridine should previously be dried over potassium hydroxide pellets, decanted, and distilled. The addition is interrupted several times to cool the contents of the bottle in an ice bath. After the addition of sulfur dioxide is complete, the resulting solution is poured into a solution of 72.5 grams of resublimed iodine in 2 liters of freshly redistilled methanol. The reagent is allowed to stand at least 24 hours before use, and then should have an initial strength of approximately 1.5 mg. of water per ml. of reagent.

Standardization of Reagent. Approximately 25 ml. of solvent are run into a flask (dried in an oven and cooled in a desiccator), and then a small excess of Fischer reagent is added to react with the water in the solvent. This excess of reagent is titrated with the water-in-methanol solution, and then a measured volume of Fischer reagent (10 to 25 ml.) is added to the dry solvent and back-titrated with the water-in-methanol solution. The yellow end point is somewhat sluggish and must be approached slowly. The color changes gradually from a red-brown to a gold-brown and then, upon the addition of 0.2 to 0.3 ml. of water-in-methanol reagent, to a brassy yellow color which is the end point. This color can be roughly approximated by a solution containing 70 mg. of potassium dichromate in 250 ml. of distilled water. Because moist air must be excluded from the flask at all times, the tip of the buret should be fitted with a rubber stopper or ground-glass joint to close the flask during the titration. A capillary tube of small diameter in the stopper will serve as a vent and still prevent diffusion of moisture into the flask. The solution may be stirred conveniently by means of a magnetic stirrer, and in this case the glass-encased agitator should be dried with the flask in an oven.

The value of R, which is the volume ratio between the Fischer reagent and the water-in-methanol solution, is then calculated. The strength of the Fischer reagent (factor F) is determined by adding to the resulting anhydrous solution in the flask approximately 20 mg. of water weighed accurately from a weighing bottle. An excess of 5 to 10 ml. of Fischer reagent is added to the flask and back-titrated with the water-in-methanol solution to the same yellow end point previously observed. The actual volume of Fischer reagent consumed by the water is calculated by utilizing ratio R. The value of F is obtained by dividing the weight of water taken (mg.) by the volume of reagent (ml.) consumed. As the reagent gradually decreases in strength, even when protected from atmospheric moisture, it should be standardized every day.

PROCEDURE

A 75-ml. portion of solvent in a carefully dried flask is made anhydrous by the addition of an excess of Fischer reagent and

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back-titration to the yellow end point with water-in-methanol solution. A further quantity, V, of Fischer reagent is then added to the anhydrous solvent in the flask in sufficient volume to be in excess of that required to react with the water in the sample. The flask is fitted with the all-glass delivery tube (the ends stoppered to exclude moisture), weighed to the nearest 0.1 gram or better, and connected to the hydrogen chloride line as illustrated in Figure 1. The flask should be placed in a salt-ice bath during the collection of the sample because a considerable amount of heat is evolved in the absorption of the hydrogen chloride.

With stopcock S closed and the bleeder line open, the valves from the hydrogen chloride supply are opened. S is then opened and the rate of flow into the sample flask is controlled by the screw clamp on the bleeder line, so that the resulting fumes in the flask do not rise more than 5 cm. above the liquid level. A small positive head of pressure prevents the solvent from backing into the line as the hydrogen chloride dissolves. At this rate of flow, a sample of 10 to 15 grams may be collected in as many minutes. When the flow of gas is interrupted the ground-glass connection joining the delivery tube to S must be quickly disconnected to prevent the liquid in the flask from backing up. The flask and delivery tube, with the ends stoppered, are reweighed to determine the amount of sample collected.

The glass delivery tube is removed and the flask is immediately closed with a ground-glass stopper. After the flask is shaken until the gas fumes are dissolved, the excess Fischer reagent is back-titrated with water-in-methanol solution. The same apparatus is used as for the initial back-titration of the excess Fischer reagent in the sample solvent, and the same end point is detected. This decreases the possibility of errors in judgment on the true end point.

After the sample has been collected, the color of the solvent must still be red-brown, for if the color has been discharged, an insufficient volume of reagent was taken to react with the water in the sample. An amount of hydrogen chloride in excess of that which can be bound by the pyridine in the solvent produces a deep red color, indistinguishable from the usual color of the Fischer reagent, which cannot be discharged even upon titrating with a large excess of water-in-methanol solution. The volume of solvent employed is sufficient to bind 18 grams of hydrogen chloride.

CALCULATIONS

Determination of water in sample.

Let V = volume (ml.) of Fischer reagent added to anhydrous solvent prior to collection of sample

U = volume (ml.) of water-in-methanol solution used in back-titration of unconsumed reagent after sample has been collected

R = volume ratio of Fischer reagent to the water-in-methanol solution

F = factor of the Fischer reagent in mg. of water per ml. of reagent

Then the milligrams of water found $= (V - U \times R)F$ and

$$\text{the per cent water found} = \frac{(V - U \times R)F}{\text{wt. of sample (grams)} \times 10}$$

Table I. Blank Determinations on Hydrogen Chloride and Hydrogen Chloride Plus Hydrocarbon Gas

Hydrogen Chloride		Hydrogen Chloride Plus Hydrocarbon Gas[a]	
Sample	H_2O found	Sample	H_2O found
Grams	*Mg.*	*Grams*	*Mg.*
2.2	0.7	2.4	1.0
3.0	0.9	3.8	0.7
5.0	0.9	4.2	0.8
5.3	0.6	4.9	1.1
5.3	0.9	7.0	1.1
Av.	0.8		0.9

[a] 0.5 to 2.0 grams of C_4 hydrocarbon gas added to each sample.

EXPERIMENTAL RESULTS

Hydrogen chloride taken from commercial cylinders of compressed gas was not satisfactory for preparing standards because of the variation in water content of the gas in different cylinders.

Table II. Analysis of Samples of Hydrogen Chloride Containing Added Water

Water Added	Water Found	Error
Mg.	*Mg.*[a]	*Mg.*
5.7	6.0	0.3
23.2	23.2	0.0
26.7	27.5	0.8
28.9	28.7	0.2
30.6	32.0	1.4
44.0	44.0	0.0
62.7	62.6	0.1
68.8	68.8	0.0
73.0	73.4	0.4
111.4	111.5	0.1
	Average deviation	0.3
	Standard deviation	0.5

[a] Corrected for blank of 0.8 mg.

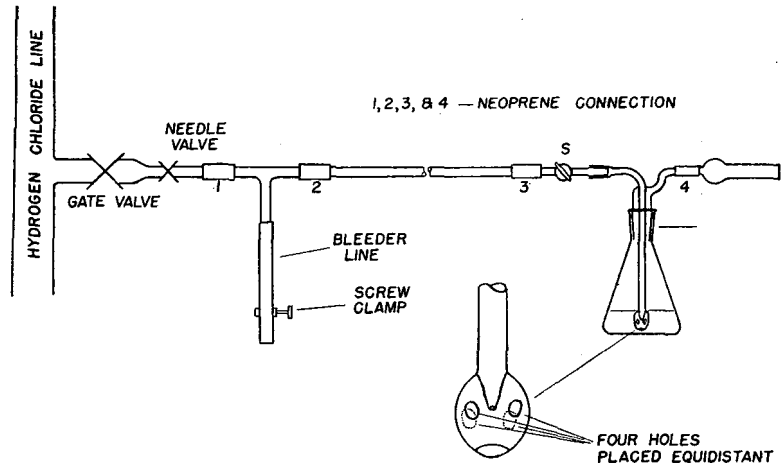

Figure 1. Apparatus for Collecting Hydrogen Chloride Sample

The hydrogen chloride could be dried satisfactorily by passing it through two scrubbers filled with sulfuric acid, but more consistent results were obtained on hydrogen chloride generated from ammonium chloride and concentrated sulfuric acid. The data in Table I show an average blank of 0.8 mg. of water, but it seems probable that the gas was anhydrous and that small amounts of water were picked up during the collection of the sample and its titration. This is substantiated by the data, which do not indicate any correlation between sample size and blank. It appears that traces of water were introduced when hydrocarbon gases were added to the hydrogen chloride. The data in Tables I, II, and III were obtained on the generated hydrogen chloride.

The results in Table II are for the analysis of samples containing known added amounts of water. This was accomplished by passing the gas through a "humidifier" containing a weighed amount of water which was vaporized with the aid of a microburner. For these analyses, the average deviation is 0.3 mg. and the standard deviation is 0.5 mg.

The presence of hydrocarbon gases in the C_4 range [*n*-butane,

Table III. Analysis of Samples of Hydrogen Chloride Containing Added Water and Hydrocarbon Gas

Water Added	Water Found	Error
Mg.	*Mg.*[a]	*Mg.*
7.6	7.8	0.2
16.2	16.2	0.0
26.0	26.1	0.1
31.1	30.9	0.2
38.4	38.5	0.1
66.1	65.5	0.6
	Average deviation	0.2
	Standard deviation	0.2

[a] Corrected for blank of 0.9 mg. Each sample contained from 0.5 to 2.0 grams of C_4 hydrocarbon.

isobutane (2-methylpropane), isobutene (2-methylpropene), 2-butene, and butadiene] was found to cause no difficulty. This is indicated by the data in Table III, which show an average deviation of 0.2 mg. and a standard deviation of 0.2 mg. The solvent action of the solution at the low temperature (−15° to −20° C.) prevented the escape of the hydrocarbon gases. If lighter hydrocarbons were present, provision would have to be made to meter the exhaust gas for inclusion in the sample weight.

The time required to conduct the complete determination is 30 to 40 minutes. This time does not include preliminary standardization of the reagent or drying of flasks and delivery tube.

A note of caution should be added on two points. During the time that the sample is being collected, the flask must be kept cold because high results have been obtained occasionally when the solution became warm. This may have been the result of reaction between the hydrogen chloride and methanol (*6*). Because hydrogen chloride is such a hygroscopic material, every precaution should be used to exclude atmospheric moisture during the analysis.

Although the samples of gas from petroleum refinery operations, for which this method was intended, will, in all probability, contain nothing but hydrogen chloride, small amounts of hydrocarbon gases, and minute amounts of water, a limited investigation of the effect of some sulfur compounds was made. As would be expected, there was no reaction with sulfur dioxide in the absence of water. However, hydrogen sulfide and methyl and ethyl mercaptans (methanethiol and ethanethiol) were oxidized in the absence of moisture.

It is well known that the following equations

$$H_2S + I_2 \longrightarrow S + 2HI$$
$$2RSH + I_2 \longrightarrow RS - SR + 2HI$$

apply in the reaction of hydrogen sulfide and mercaptans with aqueous iodine solutions. As the data in Table IV show, the same stoichiometric relationship was observed when the mercaptan and hydrogen sulfide were oxidized with the Fischer reagent. As the exact composition of the Fischer reagent was not known in terms of available iodine, the amount consumed is expressed in terms of the equivalent amount of water. On this basis, each mole of mercaptan consumes an amount of Fischer reagent approximately equivalent to 0.5 mole of water, and each mole of hydrogen sulfide is approximately equivalent to 1 mole of water. On this basis it will be possible to apply a correction for the interference of these sulfur compounds when the amounts of hydrogen sulfide and mercaptan have been determined.

Table IV. Relationship of Ethyl Mercaptan and Hydrogen Sulfide with Fischer Reagent

Wt. of Sulfur Compound *Mg.*	Iodine Consumed *Mg. H_2O*	Sulfur Compound *Mole*	H_2O *Mole*	Molar Ratio S Compound/H_2O
		Ethyl Mercaptan		
72.7	10.28	0.0012	0.0006	2/1.00
629.7	84.5	0.0101	0.0047	2/0.93
615.0	83.1	0.0099	0.0046	2/0.93
		Hydrogen Sulfide		
6.4	3.7	0.00019	0.00020	1/1.05
6.3	3.6	0.00018	0.00020	1/1.11
6.3	3.1	0.00018	0.00017	1/0.95

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Determination of Unsaturation in Dehydrogenated Dichloroethylbenzene

By Use of Mercuric Acetate

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WHEN dichlorostyrene was first considered for use in the rubber and plastic industry, no suitable analytical procedure was available for ascertaining the purity of this monomer. Owing to the presence of two chlorine atoms on the benzene ring, the addition of chemical reagents to the unsaturated side chain proceeds with considerable difficulty. Thus, the usual methods of analysis for unsaturation were found to be inadequate. The popular bromate-bromide method (Koppeschaar, *3*) and the bromination procedure using bromine in carbon tetrachloride (McIlhiney, *4*) did not give quantitative results. Low results were likewise obtained by the methods of Wijs (*14*), using iodine chloride in acetic acid; Hanus (*1*), using iodine bromide in acetic acid; and Hübl (*2*), using mercuric chloride and iodine in methanol.

A procedure previously disclosed by the authors (*6*) for the determination of the unsaturation in styrene and styrene derivatives by use of an aqueous 1,4-dioxane solution of mercuric acetate, in which the amount of mercury adding to the double bond is determined by direct titration with standard ammonium thiocyanate, gives low results with dichlorostyrene. However, it was found that mercuric acetate in methanol solution would, with moderate warming, add much more easily to carbon-carbon double bonds, and this led to the development of the following method for the quantitative estimation of the unsaturation in dichlorostyrene. It differs from the previous mercuric acetate method in that the acetic acid produced by the addition reaction is titrated instead of the mercury that chemically combines with the styrene derivative.

A new method for the determination of the terminal unsaturation in many olefinic compounds is described, in which use is made of the addition reaction of mercuric acetate to double bonds. One equivalent of acetic acid per double bond is produced and titrated. This method has proved satisfactory as an assay procedure for dichlorostyrene, styrene, and many other styrene derivatives. It is often possible to make the determination in the presence of other unsaturated or halogen-substituting compounds.

OUTLINE

Whitmore (*10*) states that mercuric salts dissolved in methanol add the groups —HgX and —OCH_3 to the double bonds of olefinic compounds. A general equation for this reaction may be shown as:

$$RCH{=}CH_2 + HgX_2 + CH_3OH \longrightarrow RCH(OCH_3)CH_2HgX + HX$$

The addition in general follows Markownikoff's rule, mercury going to the carbon having the most hydrogen atoms.

Whitmore states also that nearly all of the compounds formed from unsaturated substances and mercuric salts are soluble and stable in sodium hydroxide solution (*12*), but that they react readily with halogen acids to give back the original unsaturated compound (*11*).

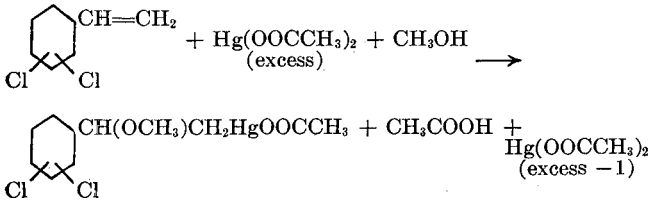
In 1919, Tausz and Peter (*9*) prepared a mercury compound of styrene by use of an aqueous mercuric acetate solution.

Manchot (*5*) found that styrene reacts with aqueous mercuric acetate to give a mercury-containing product, which was perhaps a basic compound, in which the mercury was not firmly held.

In 1928, Priewe (*8*) reported that styrene reacts with mercuric acetate in acetic acid solution to form β-phenyl-β-acetoxymercuriethyl acetate.

Wright (*15*) obtained α-acetoxymercuri-β-methoxy-β-phenylethane when he treated styrene with a methanol solution of mercuric acetate. Other workers, by reaction of styrene with an aqueous solution of mercuric acetate, produced β-acetoxymercuri-α-hydroxyethylbenzene (*7*).

In the procedure given below, a weighed sample of dehydrogenated dichloroethylbenzene reacts with an excess of mercuric acetate in methanol:



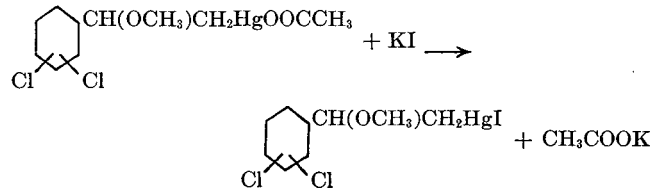
Acetone is added to the solution and the excess mercuric ion is then precipitated as mercuric oxide by the addition of a standard amount of chloride-free aqueous sodium hydroxide:

$$(CH_3COO)_2Hg + \underset{\text{(excess)}}{2NaOH} \longrightarrow HgO + 2CH_3COONa + \underset{\text{(excess }-2)}{H_2O + NaOH}$$

The mercuric oxide reacts with the acetone to produce soluble trimercuric diacetone hydrate (*13*):

$$3HgO + 2CH_3COCH_3 \xrightarrow{NaOH} HOHgCH{-}Hg{-}CHHgOH \quad (CH_3C(OH){-}O{-}C(OH)CH_3)$$

Aqueous potassium iodide is then added, which reacts with the α-acetoxymercuri-β-methoxy-β-dichlorophenylethane to form the α-iodomercuri compound, and with the trimercuric diacetone hydrate to regenerate the acetone and hydroxyl ions orginally taken by the excess mercuric acetate:



$$HOHgCH{-}Hg{-}CHHgOH \quad (CH_3C(OH){-}O{-}C(OH)CH_3) + 12\ KI + 3H_2O \longrightarrow 6KOH + 3K_2HgI_4 + 2CH_3COCH_3$$

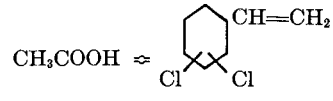
A standard amount of dilute acetic acid is added to the solution to neutralize most of the hydroxyl ions:

$$(K + Na)OH + (1 - x)\ CH_3COOH \longrightarrow (1 - x)\ CH_3COO(K + Na) + x\ (K + Na)OH$$

The remaining alkalinity is finally titrated with standard hydrochloric acid:

$$x\ (K + Na)OH + xHCl \longrightarrow x\ (K + Na)Cl + xH_2O$$

The titration obtained by use of the reagents only, or the blank titration, should be equivalent to about 49 ml. of 0.1 *N* hydrochloric acid. The acetic acid produced by the addition reaction is equivalent to the difference between the blank titration and the titration obtained by the analysis of the sample. Finally:



REAGENTS

Mercuric Acetate, approximately 0.24 *N*. Dissolve 38.0 grams of analytical reagent grade mercuric acetate in approximately 900 ml. of methanol and 2.0 ml. of glacial acetic acid. Dilute to exactly 1 liter with methanol and filter.

Acetone, good commercial grade.

Sodium hydroxide, standard 0.1 *N* (chloride-free).

Potassium Iodide Solution. Dissolve 300 grams of potassium iodide in water, add 1 ml. of 0.1 *N* sodium thiosulfate, and dilute to 1 liter. Check neutrality with phenolphthalein indicator, adjusting to a faint pink color if necessary.

Acetic Acid, approximately 0.65 *N*. Dissolve 76.0 ml. of glacial acetic acid in water and dilute to exactly 2 liters. The normality of this acid may need to be adjusted slightly to make the titration of the blank about 49 ml. of 0.1 *N* hydrochloric acid.

Hydrochloric acid, standard 0.1 *N*.

Phenolphthalein indicator.

APPARATUS

Constant-temperature bath maintained at 50° C. Two-ounce screw-cap bottles. The caps should be lined with rubber to provide a tight seal.

PROCEDURE

Pipet 50.0 ml. of the mercuric acetate solution into a screw-cap bottle and add an accurately weighed sample that will have a net titration of less than 46 ml. of 0.1 *N* hydrochloric acid. Screw the cap on tightly, mix well, and suspend the bottle in the constant-

temperature bath for 1 hour. Then take the bottle out of the bath, unscrew the cap, and pour the contents of the bottle into a 250-ml. Erlenmeyer flask, washing the bottle with methanol.

Add 10 ml. of acetone, pipet 20.00 ml. of 0.1 N sodium hydroxide into the flask, and swirl occasionally until all the mercuric oxide is dissolved. After adding 25 ml. of aqueous potassium iodide and mixing well, pipet 20.00 ml. of 0.65 N acetic acid into the flask while swirling to prevent any part of the solution from becoming acidic. Add 20 to 30 drops of phenolphthalein indicator and titrate with 0.1 N hydrochloric acid, swirling while titrating.

Finally, run a blank determination, for which the titration should be approximately 49 ml. of 0.1 N hydrochloric acid. The end point is reached when the last trace of pink color has vanished from the solution.

CALCULATION

Calculate the unsaturation for dichlorostyrene as follows:

$$\frac{A\,\frac{(\text{mol.wt.})}{10{,}000}\,100}{\text{Wt. of sample}} = \frac{A \times 0.0173 \times 100}{\text{wt. of sample}} = \%\text{ unsaturation as }\%\text{ dichlorostyrene (by weight)}$$

where

A = ml. 0.1 N hydrochloric acid (blank) − ml. 0.1 N hydrochloric acid (sample)

Table I. Analysis of Known Solutions of 2,5-Dichlorostyrene in 2,5-Dichlorodiethylbenzene

Known Number	2,5-Dichlorostyrene Calculated, %	2,5-Dichlorostyrene Found, %
	99.81 by freezing point	99.86, 100.06, 100.08, 99.82, 99.83
1	80.25	80.30, 80.31, 80.24
2	60.71	60.64, 60.74, 60.65
3	40.87	41.03, 40.98, 41.06
4	20.40	20.43, 20.41, 20.48
5	10.15	10.10, 10.22, 10.22
6	5.14	5.13, 5.18, 5.23
7	1.09	1.15, 1.14, 1.13

ADAPTABILITY OF METHOD TO STYRENE AND OTHER STYRENE DERIVATIVES

This method has been found very reliable for determining the unsaturation in styrene and in nearly all the other styrene derivatives. Accurate results are obtained because the addition reaction is quantitative and there is no possibility of substitution as in the methods involving the use of halogen. Furthermore, as styrenes in general, except those having two or more halogens on the benzene ring, react easily with mercuric acetate, it is not necessary to heat to 50° C. to complete the reaction; often 5 minutes' reaction time at room temperature will give quantitative results. Thus the mercuric acetate solution and the weighed sample may be placed directly in the 250-ml. Erlenmeyer flask.

ANALYTICAL DATA

A sample of 2,5-dichlorostyrene, 99.81% pure as determined by the freezing point method, was prepared and mixed with 2,5-dichlorodiethylbenzene in known proportions. Results obtained by analysis of these solutions with the described procedure are shown in Table I.

The results obtained on known solutions of styrene in ethylbenzene are shown in Table II. The mercuric acetate solution and the weighed samples were placed directly in 250-ml. Erlenmeyer flasks and the reaction time was limited to 5 minutes at room temperature to show that a longer time was not necessary.

It was shown in a previous paper (*6*) that mercuric acetate adds quantitatively to the vinyl groups in divinylbenzene and ethylvinyl benzene. To show the consistency of results on these two compounds over a wide range of percentages, the unsaturations in samples of ethylvinylbenzene and divinylbenzene were determined by this analytical method. Known solutions of these samples were then made with diethylbenzene and the unsaturations found by analysis were compared with the calculated values. The mercuric acetate solution and the weighed known solutions were placed directly in 250-ml. Erlenmeyer flasks and the reaction time was limited to 5 minutes at room temperature. The results obtained are shown in Table III.

Table II. Analysis of Known Solutions of Styrene in Ethylbenzene

Known Number	Styrene Calculated, %	Styrene Found, %
	99.60 by freezing point	99.46, 99.74, 99.38, 99.50, 99.51
1	75.71	75.52, 75.44, 75.50
2	50.87	50.63, 50.80, 50.76
3	20.57	20.52, 20.52, 20.51
4	1.05	1.06, 1.06, 1.05

Table III. Analysis of Solutions of Ethylvinylbenzene, Divinylbenzene, and Diethylbenzene

Known Number	EVB Added (Calculated)	DVB Added (Calculated)	Total Unsaturation (Calculated)	Total Unsaturation (Found)
			Per Cent as Ethylvinylbenzene	
EVB				98.79, 98.78, 98.74
DVB				201.0, 200.6, 200.7
1	168.22	16.02	184.2	184.0, 184.1, 184.0
2	90.44	42.92	133.3	133.4, 133.0, 132.9
3	41.95	39.57	81.52	81.39, 81.21, 81.11
4	11.86	10.64	22.50	22.54, 22.54, 22.47
5	3.07	2.52	5.59	5.77, 5.78, 5.77
6	0.99	0.48	1.47	1.63, 1.66, 1.70

Other styrene derivatives that have been successfully analyzed by this method are vinyltoluene, vinylxylene, and ethoxystyrene (5 minutes' reaction time at room temperature), and monochlorostyrene and vinylchlorotoluene (15 minutes' reaction time at room temperature). Experience seems to indicate that the method gives somewhat low results with α-methylstyrene, because perhaps the addition compound is not completely stable in basic solution in the presence of potassium iodide.

DISCUSSION

Because mercuric acetate dissolved in methanol adds easily and quantitatively to the vinyl group attached to the benzene ring, the analytical procedure described is generally well suited to the analysis of the unsaturation in styrene and styrene derivatives. There is no danger of substitution to cause high results.

In general, mercuric acetate in methanolic solution adds readily and quantitatively to compounds containing terminal unsaturation, such as allylbenzene, but slowly and not quantitatively to compounds containing nonterminal unsaturation, such as propenylbenzene, thus limiting the method to the analysis of the double bonds in styrene, styrene derivatives, and many end-of-chain unsaturated compounds. This selective feature has been used, with proper limitations of reaction time and temperature, to determine directly the unsaturation of compounds containing terminal double bonds in the presence of other unsaturated compounds that react with halogen but not with mercuric acetate under the conditions of the analytical procedure.

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Laboratory Low Temperature Fractional Distillation

Optimum Distillation Rates and Fraction Cut Points

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In a previous paper (4) the results of a study of optimum charging rates were reported. The present paper contains the results of a continuation of this study in which optimum distillation rates and fraction cut points have been determined. Two samples were employed: one was a plant stream containing appreciable amounts of noncondensables and C_1, C_2, C_3, C_4, and C_5 hydrocarbons, and the other contained the same components, but the C_3 was present in only minute quantity. After charging and refluxing for a proper amount of time, the distillations were made at several different rates, and the heart cut of each fraction was analyzed by mass spectrometer for contamination by lower and higher boiling constituents. Analyses were also made of total major fractions and fractions taken between major boiling points to determine if generally accepted cut points were optimum. It has been found that distillation rates as high as 100 cc. per minute may be used with negligible amount of contamination. If the column is operated properly, the amount of hydrocarbons distilling between boiling points is small and the contamination of one fraction with another, using generally accepted cut points, is negligible. The contamination is usually due to the lower boiling fraction.

IN A previous paper (*4*) on low temperature distillation studies, the results of an investigation of optimum charging rates were reported. In that study it was shown that the precision for the determination of major fractions was only slightly different for very fast distillation rates than for the slower rates recommended in the literature (*1*, *2*). The precision obtained at high distillation rates could be due to compensating effects of contamination of each fraction by the next higher and lower boiling components. The present study was initiated to determine the extent to which this phenomenon occurred under different conditions and to determine optimum conditions for minimizing these effects.

In the usual complete analysis of gases employing low temperature distillation, the mixture is charged to the distillation column, which is maintained at a low temperature by the use of liquid nitrogen, and the noncondensable portion is taken overhead, measured, and submitted to chemical analysis for determination of individual constituents. The remaining condensed portion is fractionally distilled, and groups of hydrocarbons containing the same number of carbon atoms and boiling in the same range are removed together for subsequent analysis for individual constituents. As chemical and physical methods for the analyses of these fractions generally only identify types of compounds, such as saturates, unsaturates, etc., it is necessary that contamination of one fraction with that of another of different carbon number be very small in order to obtain accurate results for individual components.

In the present study, two gas samples have been distilled at several different rates and the fractions analyzed to determine the extent of contamination of one fraction by another. Portions taken overhead between major boiling points have been analyzed to determine the optimum cut points to be employed.

EQUIPMENT AND SAMPLES

The equipment used in this study consisted of a Podbielniak Hyd-Robot distillation apparatus (*3*), a Consolidated Engineering Corporation mass spectrometer (*5*), special sampling bottles, and conventional laboratory equipment. Two gases were employed in this study. Sample 1 was a product gas stream from a fluid catalyst cracking unit, and its approximate composition was 14% noncondensable, 34% methane, 12% C_2, 22% C_3, 15% C_4, and 3% C_5. Sample 2 contained approximately 15% hydrogen, 15% methane, 10% C_2, less than 1% C_3, and 60% C_4. This gas was analyzed to determine the contamination to be expected of a gas where one of the major components was either missing or present in only minute quantities, causing a large spread in boiling points between succeeding fractions. The samples were retained in a 25-gallon cylinder at a pressure below the dew point of the heavier hydrocarbons.

GENERAL DISTILLATION PROCEDURE

The column was charged according to the procedure outlined in a previous paper (*4*). When sufficient sample was condensed in the still pot, the entering line and stopcock were sealed with mercury, and the pot heater was turned on and set to deliver 2 watts of heat. The excess liquid nitrogen around the still and in the column was removed by blowing air up the column until all of the liquid nitrogen was removed.

After the removal of the fixed gases from the column and still, indicated by approximately 150 cc. overhead on the methane plateau, the column was placed under total reflux until the receivers could be changed. The amount of heat supplied to the still while taking overhead on a plateau was sufficient to maintain the indicated rate of take-off and, at the same time, to require reflux cooling at 8- to 10-second intervals. As the column was under total reflux while changing receivers at the cut points, the heat was removed to prevent flooding.

The distillation rate was automatically slowed a short distance before the cut point and did not exceed 3 to 4 cc. per minute during the "break" from one plateau to another.

DISTILLATION RATE STUDY

Determination of Precision of Total Fraction Analysis. The nominal distillation rates employed were 25, 50, and 100 cc. per minute. Fractions were segregated employing the usual cut points—i.e., C_1–C_2 at $-120°$ C., C_2–C_3 at $-60°$ C., C_3–C_4 at $-27°$ C., and C_4–C_5 at $+18°$ C. (or $-6°$ C. at 300-mm. absolute pressure). The fractions were analyzed by mass spectrometer, and the results are shown in Table I.

The data shown in Table I indicate the amount of contamination of each fraction by the next higher and lower boiling components when using the indicated distillation rates and the usual cut points described above. In general, the major portion of the contamination is due to the lower boiling components which have not been completely removed in the previous fraction. Although this contamination amounts to as much as 2 to 3% in the large

Table I. Effect of Distillation Rate on Total Fraction Contamination

	Rate dist. cc./min.	Mole % of Fraction (M.S. Analysis)							Mole % of Total Sample						
Sample 1															
C_1 fraction		[a]	CH_4	C_2H_6					[a]	CH_4	C_2H_6				
48% (34% CH_4, 14% non-condensables)	25		100.00	...						33.60	...				
	25		100.00	...						35.35	...				
	50		99.92	0.08						34.77	0.03				
	50		100.00	...						34.90	...				
	100		100.00	...						32.90	...				
	100		100.00	...						35.00	...				
C_2 fraction		CH_4	C_2H_4	C_2H_6	C_3H_6	C_3H_8			CH_4	C_2H_4	C_2H_6	C_3H_6	C_3H_8		
12%	25	1.12	34.22	64.63	0.03	...			0.12	3.76	7.12	...	...		
	25	1.40	35.10	63.50	...	...			0.17	4.14	7.49	...	...		
	50	1.12	34.53	63.94	0.33	0.08			0.13	4.07	7.55	0.04	0.01		
	50	1.65	34.11	64.12	0.12	...			0.19	4.02	7.58	0.01	...		
	100	1.95	32.92	64.89	0.24	...			0.23	3.88	7.66	0.03	...		
	100	1.35	34.30	64.11	0.24	...			0.16	4.01	7.50	0.03	...		
C_3 fraction		C_2H_4	C_2H_6	C_3H_6	C_3H_8	Iso-C_4H_{10}	C_4H_8		C_2H_4	C_2H_6	C_3H_6	C_3H_8	Iso-C_4H_{10}	C_4H_8	
22%	25	...	0.73	56.16	43.05	0.02	0.05		...	0.16	12.59	9.64	...	0.01	
	25	...	0.81	55.81	43.28	0.04	0.06		...	0.18	12.39	9.61	0.01	0.01	
	50	0.35	0.31	54.47	44.80	0.07	...		0.08	0.07	12.04	9.90	0.02	...	
	50	0.02	0.80	55.54	43.60	...	0.04		...	0.18	12.16	9.55	...	0.01	
	100	...	0.45	56.23	43.07	0.04	0.21		...	0.10	12.30	9.44	0.01	0.05	
	100	0.47	0.78	56.27	41.80	...	0.68		0.10	0.17	12.38	9.20	...	0.15	
C_4 fraction		C_3H_6	C_3H_8	Iso-C_4H_{10}	n-C_4H_{10}	C_4H_8	C_5H_{10}	C_5H_{12}	C_3H_6	C_3H_8	Iso-C_4H_{10}	n-C_4H_{10}	C_4H_8	C_5H_{10}	C_5H_{12}
15%	25	No M.S. analysis													
	25	0.07	0.94	33.73	7.97	57.17	...	0.12	0.01	0.14	5.09	1.20	8.64	...	0.02
	50	...	0.71	29.20	7.47	62.08	0.11	0.43	...	0.11	4.41	1.13	9.37	0.02	0.06
	50	0.12	1.20	31.74	8.10	58.55	0.10	0.19	0.02	0.18	4.79	1.22	8.84	0.02	0.03
	100	...	1.34	29.84	6.64	61.36	0.29	0.53	...	0.20	4.54	1.01	9.93	0.04	0.08
	100	...	2.90	28.05	7.31	61.52	0.07	0.15	...	0.44	4.24	1.12	9.35	0.01	0.02
C_5 fraction		C_4H_8	C_5H_{10}	Iso-C_5H_{12}	n-C_5H_{12}	C_6+			C_4H_8	C_5H_{10}	Iso-C_5H_{12}	n-C_5H_{12}	C_6+		
3%	25	No M.S. analysis							...	...	...	...	...		
	25	8.98	52.16	34.41	4.45	...			0.27	1.56	1.03	0.13	...		
	50	No M.S. analysis							...	...	...	...	...		
	50	2.76	58.40	34.98	3.86	...			0.09	1.93	1.15	0.13	...		
	100	2.44	55.87	37.19	4.42	0.08			0.08	1.84	1.23	0.15	...		
	100	1.96	57.21	36.23	4.44	0.16			0.07	2.06	1.30	0.16	0.01		
Sample 2															
C_1 fraction		[a]	CH_4	C_2H_4					[a]	CH_4	C_2H_4				
30% (15% CH_4, 15% H_2)	25		100.00	...						13.0	...				
	50		100.00	...						13.8	...				
	100		99.93	0.07						11.1	0.01				
C_2 fraction		CH_4	C_2H_4	C_2H_6	C_3H_6				CH_4	C_2H_4	C_2H_6	C_3H_6			
10%	25	1.85	77.64	20.43	0.08				0.19	7.92	2.08	0.01			
	50	1.36	78.44	20.09	0.11				0.14	7.92	2.03	0.01			
	100	2.44	81.14	16.34	0.08				0.24	7.95	1.60	0.01			
C_4 fraction		C_2H_4	C_2H_6	C_3H_8	Iso-C_4H_{10}	n-C_4H_{10}	C_4H_8		C_2H_4	C_2H_6	C_3H_8	Iso-C_4H_{10}	n-C_4H_{10}	C_4H_8	
60%	25	0.19	0.36	0.90	13.58	41.01	43.96		0.12	0.22	0.56	8.41	25.39	27.20	
	50	0.05	0.13	0.49	12.55	42.94	43.84		0.03	0.08	0.31	7.86	26.88	27.44	
	100	0.09	0.30	0.28	13.08	38.94	47.31		0.06	0.19	0.18	8.23	24.49	29.75	

[a] Contamination of methane fraction with lower boiling noncondensables was disregarded, as these components are not fractionally separated in Podbielniak column under normal operating conditions.

fractions and 9% in the small fraction (C_5), the resultant error in the analysis on a total sample basis is small. However, the accuracy of the mass spectrometer for the lighter components in some of these cases is in the order of 1 to 2% of the total fraction.

In the case of sample 2 a considerable quantity of C_3 is shown in the analysis of the C_4 fraction. No C_3 was indicated from the distillation curve. This apparent anomaly may be due in part to the limitation of the distillation equipment and in part to the mass spectrometer analysis of the fraction.

The effect of distillation rate on the amount of contamination shows no definite trend, and it is indicated that the highest rate (100 cc. per minute) may be used on gases without any appreciable loss in accuracy. In general, the effect of these contaminants on the

Table II. Effect of Distillation Rate on Purity of Heart Cut

(Sample 1)

	Rate Dist., Cc./Min.	Mole % of Heart Cut Fraction (M.S. Analysis)					Mole % Contaminant Total Sample Basis		
C_1 fraction		[a]	CH_4	C_2H_4			C_2H_4		
	25		100.00	...			...		
	25		100.00	...			...		
	50		100.00	...			...		
	50		99.96	0.04			0.01		
	50		100.00	...			...		
	100		99.96	0.04			0.01		
	100		100.00	...			...		
C_2 fraction		CH_4	C_2H_4	C_2H_6	C_3H_6	C_3H_8	CH_4	C_3H_6	C_3H_8
	25	0.10	33.91	65.70	0.27	0.02	0.01	0.03	0.00
	25	0.22	44.39	55.39	...	...	0.02	...	...
	50	0.10	41.80	57.42	0.68	...	0.01	0.06	...
	50	0.15	47.09	52.01	0.75	...	0.01	0.06	...
	50	0.15	40.49	58.79	0.57	...	0.02	0.06	...
	100	0.17	46.13	53.20	0.35	0.15	0.02	0.03	0.01
	100	0.12	44.57	54.91	0.40	...	0.01	0.04	...
C_3 fraction		C_2H_4	C_2H_6	C_3H_6	C_3H_8	Total C_4	C_2H_4	C_2H_6	Total C_4
	25	...	...	65.58	34.36	0.06	...	...	0.01
	25	...	...	74.86	25.14	...	...	...	...
	50	...	0.52	72.48	26.26	0.74	...	0.11	0.15
	50	0.30	...	83.89	15.56	0.25	0.05	...	0.04
	50	0.12	...	83.83	15.56	0.49	0.02	...	0.08
	100	0.14	...	76.33	23.53	...	0.02	...	...
	100	0.43	0.19	72.93	25.93	0.52	0.07	0.03	0.09

[a] Contamination of methane fraction with lower boiling noncondensables was disregarded, as these components are not fractionally separated in Podbielniak column under normal operating conditions.

further analyses of each fraction by present chemical or spectroscopic methods would be approximately the same or less than the amounts of contaminant shown.

Determination of Purity of Heart Cut. In order to determine the purity of the fractions at the several distillation rates, heart cut samples were taken of each fraction after about 50 cc. of gas had been taken overhead and before the next cut point was reached. This ensured determination of the purity of the fraction without regard to contamination due to error in cut point. These fractions were analyzed by mass spectrometer, and the results are shown in Table II (Sample 1).

No trend in contamination of the heart cuts may be seen with increase of distillation rate, and the quantities of contaminant in each case are small.

In both studies, precision of total fraction analysis and purity of heart cut, it was necessary to place the column under total reflux for several minutes while changing receivers to accommodate the indicated fractions. Although this was not desirable in a study of the effect of distillation rates, it was necessary because of the nature of the apparatus employed.

DETERMINATION OF OPTIMUM CUT POINTS

In this study, the distillation was allowed to proceed at a predetermined rate (25 cc. per minute) until the temperature of the gas taken overhead from the distilling column began to rise above the boiling point of the highest boiling component in the fraction. At this point the overhead gas line was switched to a manifold containing 15-cc. capacity receivers filled with mercury, and the mercury was slowly displaced by the effluent gas. Employing this procedure, it was possible to segregate fractions without placing the distillation column under total reflux at any critical point in the distillation. The boiling range of each of these small fractions was recorded and the gas analyzed by mass spectrometer. As each of the distillations reported in this paper employed approximately 5000 cc. of gas, these specially collected fractions represented only a few tenths of 1% of the total gas. The results of the analyses of these fractions are given in Table III.

It was necessary to collect equal quantities of each of the small fractions in order to accumulate sufficient amounts for mass spectrometer analysis. Accordingly, none of the boiling ranges shown in Table III corresponds exactly to the normal cut points. However, horizontal dotted lines indicate for each fraction the point that most nearly approximates the normal cut point. Values for the lighter component falling below this line indicate the amount of "carry-over" of this component into the next fraction and values of the heavier components above this line indicate the amount of contamination of the previous fraction with the heavier fraction. In each case, this amount is small when determined on the total sample basis. Although it has generally been considered that the first fraction analysis would be low and the last fraction high by the same amount, owing to holdup of the column

Table III. Analysis of Cut Point Fractions[a]

(Sample 1)

Cut Point	Fraction	Temperature Range, ° C.	Mole % of Fraction (M.S. Analysis)				Mole % of Total Sample			
			First Distillation							
			CH_4	C_2H_4	C_2H_6		CH_4	C_2H_4		
C_1–C_2	1	−104 to −104	35.21	61.02	0.78		0.09	0.16		
	2	−104 to −104	4.91	91.60	0.81		0.01	0.25		
	3	−104 to −104	0.69	96.21	0.49		...	0.26		
	4	−104 to −104	...	99.79	...		...	0.27		
			C_2H_4	C_2H_6	C_3H_6	C_3H_8		C_2H_6	C_3H_6	
C_2–C_3	1	−88 to −52		No M.S. analysis						
	2	−52 to −49	0.79	64.61	33.21	1.27		0.17	0.10	
	3	−49 to −49	0.57	13.99	82.91	2.03		0.04	0.20	
	4	−49 to −48		No M.S. analysis				...	...	
			C_3H_8	C_3H_8	Iso-C_4H_{10}	C_4H_8	C_3H_8	C_3H_8	Iso-C_4H_{10}	C_4H_8
C_3–C_4	1	−40 to −14	9.19	73.69	15.80	1.32	0.02	0.20	0.04	...
	2	−14 to −12	2.83	22.89	69.53	4.70		0.06	0.19	0.01
	3	−12 to −12	2.81	7.25	85.48	4.46		0.05	0.23	0.01
	4	−12 to −12	1.03	2.51	90.81	5.65		...	0.25	0.02
			n-C_4H_{10}	n-C_4H_8	Iso-C_5H_{12}	C_5H_{10}		n-C_4H_8	Iso-C_5H_{12}	C_5H_{10}
C_4–C_5[b]	1	−15 to −10	4.53	89.06	3.78	2.27		0.08	...	...
	2	−10 to −5	3.49	82.16	8.94	4.19		0.08	0.01	...
	3	−5 to +1	4.29	68.74	14.04	9.68		0.06	0.01	0.01
	4	+1 to +4	0.97	43.14	41.34	13.22		0.04	0.04	0.01
			Second Distillation							
			CH_4	C_2H_4	C_2H_6		CH_4	C_2H_4		
C_1–C_2	1	−150 to −145	99.41	0.38	...		0.27			
	2	−145 to −115	98.39	1.37	0.10		0.27			
	3	−115 to −104	46.49	52.35	...		0.13	0.14		
	4	−104 to −104	3.70	95.69	0.30		0.01	0.26		
			C_2H_4	C_2H_6	C_3H_6	C_3H_8		C_2H_6	C_3H_6	
C_2–C_3	1	−80 to −58	1.97	79.42	16.69	0.60		0.21	0.04	
	2	−58 to −50	0.70	73.87	23.60	0.60		0.20	0.06	
	3	−50 to −48	0.42	19.64	77.59	1.31		0.05	0.21	
	4	−48 to −48	0.09	4.01	93.75	1.45		0.01	0.25	
			C_3H_6	C_3H_8	Iso-C_4H_{10}	C_4H_8		C_3H_8	Iso-C_4H_{10}	
C_3–C_4	1	−42 to −30	0.74	97.54	1.40	0.12		0.26	...	
	2	−30 to −18	0.90	89.52	9.19	0.39		0.24	0.02	
	3	−18 to −13		64.56	33.13	2.11		0.17	0.10	
	4	−13 to −12		15.71	80.45	3.36		0.04	0.23	
			n-C_4H_{10}	n-C_4H_8	Iso-C_5H_{12}	C_5H_{10}		n-C_4H_8	Iso-C_5H_{12}	C_5H_{10}
C_4–C_5[b]	1	−20 to −2	0.73	64.91	24.68	7.70		0.06	0.02	0.01
	2	−2 to +2	1.33	43.96	41.38	13.07		0.04	0.04	0.01
	3	+2 to +4	0.10	28.79	53.23	15.59		0.03	0.05	0.01
	4	+4 to +4	0.15	22.19	59.30	16.76		0.02	0.06	0.01

[a] Horizontal dotted lines indicate nearest approximation to normal cut point.
[b] Distillation column pressure 300 mm. absolute.

Table II. (*Cont'd*)

(Sample 1)

C_4 fraction	Rate Dist., Cc./Min.	Mole % of Heart Cut Fraction (M.S. Analysis) C_3H_8	C_4H_8	n-C_4H_{10}	Iso-C_4H_{10}	Mole % Contaminant Total Sample Basis C_3H_8
	25		No M.S. analysis			...
	25	0.53	39.38	10.46	49.63	0.07
	50	...	38.34	7.53	54.13	...
	50	...	32.66	1.51	65.83	...
	50	...	37.94	5.38	56.68	...
	100	...	45.16	9.68	45.16	...
	100	...	45.91	9.39	44.70	...
C_5 fraction		C_4H_8	n-C_5H_{12}	Iso-C_5H_{12}	C_5H_{10}	C_4H_{10} C_4H_8 C_4H_{10}
	25		No M.S. analysis			... ...
	25		No M.S. analysis			... ...
	50	0.83	6.87	46.61	45.69	... 0.02 ...
	50	3.65	1.24	76.66	17.80	0.65 0.05 0.00
	50	5.44	1.95	69.69	22.15	0.77 0.10 0.01
	100	2.95	2.11	68.71	25.88	0.35 0.06 0.01
	100	2.35	3.66	61.21	32.06	0.72 0.05 0.02

[a] See opposite page for footnote to table.

as well as the lines beyond the point at which the temperature is measured, only a very slight trend in this direction is shown by the results in Table III. As the fractions analyzed represent only a few tenths of 1% of the total sample, the results shown in Table III should be accurate on a total sample basis.

CONCLUSIONS

The results obtained show that distillation rates as high as 100 cc. per minute may be employed with accuracy equal to that attained at the slower rates prescribed in the literature. Cut points between fractions could be raised somewhat over those previously prescribed with a resulting very slight increase in accuracy.

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Water-Soluble Polycarboxylic Acids from Oxidation of Bituminous Coal

Determination of Molecular Weights

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The application of modifications of the Menzies-Wright molecular weight apparatus to the determination of the molecular weights of the water-soluble polycarboxy acids formed by oxidation of coal is described. Acetone and methyl ethyl ketone were used as solvents and benzoic, salicylic, phthalic, and mellitic acids as standard solutes. For solutes that obey Raoult's law there is a linear relation between the rise in the differential thermometer and the millimoles of solute present, if a constant volume of solvent is added to the apparatus and a constant energy input to the boiler maintained. This constant relating the rise in the water thermometer and molar concentration combines the classical ebullioscopic constant and the coefficient of the water thermometer, and greatly simplifies calculations. This constant is affected by changes in barometric pressure, about 1.6% for each 10 mm., and deviations from Raoult's law are shown by departure from linearity at higher concentrations. No such deviations greater than experimental error were observed with these solutes in the ketonic solvents used.

CONTROLLED oxidation of suspensions of bituminous coal in aqueous alkali by oxygen gas, at elevated temperatures and pressures, results in a mixture of water-soluble carboxylic acids in yields as high as 60% by weight of the coal (*1*). Simple aliphatic acids, acetic and oxalic, are formed in small amounts, but aromatic polycarboxy acids predominate. Benzene di-, tri-, tetra-, and pentacarboxylic acids have been isolated and there is evidence of the presence in significant amounts of acids of more complex nucleus than the benzene ring.

Because the acids differ both as to nuclear size and number of functional groups per molecule (equivalent weight) it is necessary, in following the effects of process variables and fractionation procedures, to have available rapid methods for determination of both equivalent and molecular weights. Determination of the former presents no difficulty, inasmuch as we are dealing with strong water-soluble acids, the equivalent weights of which are readily determined by the usual titrimetric procedures. For the molecular weight determinations the Menzies-Wright apparatus (*5, 6*) as modified in this laboratory and by Hanson and Bowman (*2*) has been found satisfactory from the standpoint of convenience, speed, and reproducibility. Data on four known solutes—benzoic, phthalic, salicylic, and mellitic acids—as well as on samples of the mixed aromatic acids recovered from the oxidation of coal, are presented. Acetone and methyl ethyl ketone were used as solvents. A simple method for establishing the constant for calculation of the results is described.

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REAGENTS AND APPARATUS

The acetone and the benzoic and salicyclic acids were Mallinckrodt analytical reagent grade. The acetone was used without further purification. The phthalic acid (Eastman) was crystallized once from water. The mellitic acid was prepared by oxidation of carbon black; the crude acid was precipitated as the ammonium salt and the free acid recovered by electrolysis of an aqueous solution of the salt. The equivalent weight, by titration with 0.1 *N* alkali using phenolphthalein indicator, was found to be 57.3; theoretical, 57.0. All the acids were dried 2 hours at 110° C. before being used.

The methyl ethyl ketone was a commercial product fractionated through an 8-plate column, packed with 0.125-inch stainless steel helices, at a 20 to 1 reflux ratio. The cut used boiled between 78.5° and 78.8° C. (uncorrected).

Two modifications of the apparatus were employed. The first was the original type of Menzies-Wright apparatus without vacuum jacket and with a removable Cottrell pump, modified for electric heating by the introduction of a helical coil of No. 18 Nichrome wire. The external diameter of the helix was such as to allow the mouth of the Cottrell pump to pass over it and the ends were brazed to 2-mm. tungsten leads sealed through the glass. The total resistance of heater and leads was about 0.8 ohm and the electrical energy for heating was supplied through a small transformer. The second modification was a recent model of the

Table I. Relation between Barometric Pressure and Water Thermometer Coefficients

P, Mm. Hg	T, ° C.	Differential, Water, Thermometer (5, 6), Mm./° C.
	Acetone	
760	56.141 (8)	81.77
750	55.754	80.52
745	55.558[a]	79.85
740	55.362	79.18
730	54.967	77.88
	Methyl Ethyl Ketone (7)	
760	79.57	198.2
750	79.16	195.4
745	78.95[a]	194.0
740	78.75	192.6
730	78.34	189.9

[a] Interpolated.

Hanson-Bowman apparatus, supplied with vacuum jacket, fixed Cottrell pump, and radiant heater, drawing about 8 amperes at 2.5 volts. The wattage input varied ±2% and within this variation no significant effects on the readings of the differential thermometer were observed.

PROCEDURE

Because of the known wide deviations of carboxylic acids from Raoult's law in hydrocarbon solvents, only ketonic solvents were used.

After the zero reading of the thermometer was obtained in the usual way (2), the solute, when nonhygroscopic and readily pelleted, was added in increments to a known volume of the solvent. With solutes not adapted to pelleting, solutions of the required concentrations were prepared outside the apparatus and the appropriate volume of solution was added.

In methods for the ebullioscopic determination of molecular weights the problem of establishing the concentration of the boiling solution enters. If, however, standardization is carried out with solutes of known molecular weight, and if the ratio of solvent in the solution to the total added remains constant at all times, the difficulty is obviated (4). Reasonable constancy in this respect can be attained by a constant energy input to an electric heater and some care as to rate of flow and temperature of condensing water. This procedure, which has been followed in the present work, results in a very simple relation between the observed rise on the water thermometer and the molar concentration of the solution, $\Delta h = km$, where Δh is the rise and m is molar concentration. This relation holds, for a solute obeying Raoult's law, because over the short temperature intervals with which we are concerned the change in vapor pressure of the water in the thermometer with temperature is linear. The constant, k, conveniently expressed as millimeter per millimole of solute in 25 ml. of added solvent, combines the classical ebullioscopic constant for the particular solvent employed with the temperature coefficient of the water thermometer. The use of this combined constant greatly simplifies calculations in molecular weight determinations.

This constant is somewhat affected by changes in barometric pressure, about 1.6% decrease for each 10-mm. decrease in barometric pressure, and the exact magnitude of the effect can be calculated from boiling point temperature data for the solvent employed and the temperature coefficients of the water thermometer. The required data for acetone and methyl ethyl ketone are given in Table I. Thus, for example, if the constant determined when the barometric pressure is 750 mm. is found to be 7.70 mm. per millimole in 25 ml. of added acetone, then with a barometric pressure of 740 mm., since the relations are linear, the constant will be $\frac{79.18}{80.52} \times 7.70 = 7.57$.

RESULTS AND DISCUSSION

Data typical of the results with benzoic acid in acetone in the Type 2 apparatus are shown in Table II. It is evident that the degree of reproduciblity is satisfactory and that there is no systematic change in $\Delta h/m$ over the concentration range used; the highest concentration is about 3 mole %. Evidently deviations from Raoult's law for this system, over this concentration range, are less than the experimental error. Positive deviations from Raoult's law (association) would be shown by decreasing values of $\Delta h/m$ with increasing concentration.

In Table III are given minimum, maximum, and average values of $\Delta h/m$ for salicylic, phthalic, and mellitic acids. These represent results of experiments in which concentrations of 1 to 10 millimoles of solute in 25.0 ml. of acetone were used. The average values of $\Delta h/m$ corrected to a barometric pressure of 745 mm., a common value in this district, are also shown and it is evident that the use of the pressure correction improves the agreement. The average value of k for the four standard solutes, at 745 mm., is 7.55 and this value was used in the determinations on the mixed acids.

Satisfactory but less exactly reproducible results were obtained with the older Type 1 apparatus and using methyl ethyl ketone as a solvent. The difficulty probably lies in both the apparatus and the lower purity of the solvent. It was not found possible to use methyl ethyl ketone in the refined Type 2 apparatus because not enough energy could be supplied through the radiant heater without exceeding the rated capacity. The very much greater

Table II. Relation between Moles of Solute and Δh

Solvent, 25.0 ml. of acetone P = 744 mm. of Hg
Solute, benzoic acid E = 19.2 watts

Solute, millimoles	1.887	2.808	4.038	5.760	7.164	8.411	9.573	10.381
Δh	14.4	21.1	30.4	43.2	54.4	63.5	72.3	78.7
$\Delta h/m$	7.62	7.52	7.52	7.50	7.60	7.55	7.55	7.58
							Av.	7.55
							At 745 mm.	7.60

Table III. Values of $\Delta h/m$ for Salicylic, Phthalic, and Mellitic Acids

(25.0 ml. of acetone used in each instance)

Solute	Barometric Pressure, Mm. Hg	$\Delta h/m$ Min.	$\Delta h/m$ Max.	$\Delta h/m$ Av.	$\Delta h/m$ (at 745 Mm.)
Salicylic acid	730	7.22	7.47	7.33	7.48
Phthalic acid	740	7.38	7.61	7.46	7.52
Mellitic acid	749	7.60	7.90	7.72	7.65

Table IV. Molecular Weight of Mixed Aromatic Acids from Coal

Solvent, 25.0 ml. of acetone P = 744 mm. of Hg

Solute, Grams	Differential Thermometer Readings: Left arm, L, cm.	Right arm, R, cm.	$L - R$	Δh, Mm.	Molecular Weight[a]
0.0	2.14	1.16	0.98		
0.5103	3.49	0.98	2.51	15.3	251
0.5832	3.70	0.96	2.74	17.6	250
0.6923	3.96	0.92	3.04	20.6	253
0.8445	4.40	0.87	3.53	25.5	249
1.027	4.90	0.82	4.08	31.0	249
					Av. 250

[a] Calculated from the relation:

$$\text{Molecular weight} = \frac{K \times g \times 1000}{\Delta h}$$

where K = 7.54, g = weight of solute, and Δh = rise in differential thermometer.

Table V. Molecular Weights of Fractionated Acids

Fraction	Weight, Grams	Molecular Weight	Moles	Mole %
1	463	199	2.32	32.0
2	499	235	2.12	29.2
3	284	256	1.11	15.3
4	97	280	0.34	4.7
5	39	326	0.12	1.6
6	17	374	0.04	0.5
R	535	442	1.21	16.7
	1934		7.26	100.0

$$\text{Number average Molecular weight} = \frac{1934}{7.26} = 266$$

sensitivity of the water thermometer with the higher boiling solvent, more than twice the rise per millimole, emphasizes the importance of having a differential thermometer filled with a lower boiling liquid than water if low boiling solvents such as acetone are to be employed. Differential thermometers filled with a number of different liquids have recently been described (*3*).

The data of Tables IV and V show the application of the method to the mixed acids recovered from the oxidation of coal. Those in Table IV refer to a typical unfractionated mixture such as is recovered from the pilot plant operations and those in Table V illustrate the use of the method in following fractional separation by solvents. In this separation about 2 kg. of the mixed acids, of average molecular weight 250, were subjected to a fractionation process by ether-pentane mixtures. The weights of the fractions recovered and the average molecular weights found for each fraction are shown. From these data the "number average" molecular weight is calculated. In view of recovery losses and the difficulty of complete elimination of solvents, the agreement of the number average, calculated from the fractionation data, with the value for the original mixture, is satisfactory and lends confidence to the values for both the original mixture and the fractions.

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Determination of Organic Hydrazines

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A procedure is described for determining organic hydrazines by oxidation with cupric sulfate and measurement of the liberated nitrogen.

SEVERAL oxidants have been used in determining hydrazines and hydrazine salts: potassium iodate (*5, 6, 9*), potassium permanganate (*4, 6, 9*), potassium bromate (*10*), iodine (*6, 9*), calcium hypochlorite (*11*), chloramine T (*8*), potassium ferricyanide (*7*), ceric salts (*3*), and cupric ion (*1, 2*). The amount of hydrazine was usually determined by measuring the oxidant consumed. When potassium ferricyanide was used as the oxidant (*7*), the nitrogen evolved was collected and measured.

In attempting to use the above oxidants to determine hydrazines of the type $RNHNH_2$, it was found that the reaction was slow, and a quantitative amount of the oxidant was not consumed. Heat caused undesirable side reactions such as the oxidation of side chains, but, although the warm oxidation proceeded in indeterminate manner, the nitrogen was liberated quantitatively from the hydrazine. A nitrometric method based upon these observations has, therefore, been developed.

Potassium iodate, potassium permanganate, and ceric sulfate were tried as oxidants but were found to have disadvantages. Iodine was liberated from the potassium iodate which sublimed through the apparatus and into the nitrometer. Ceric sulfate and potassium permanganate caused the reaction to proceed in an indeterminable manner. Cupric sulfate in sulfuric acid oxidized the hydrazine quantitatively and could be easily handled in the apparatus.

The oxidation for the monosubstituted hydrazines proceeds according to the following reaction:

$$RNHNH_2,H_2SO_4 \xrightarrow[[2O]]{CuSO_4} RN{\equiv}N^+ HSO_4^- + 2H_2O \xrightarrow[H_2O]{Heat} ROH + N_2 + H_2SO_4$$

The reaction mechanism for the more highly substituted hydrazines is uncertain, but the nitrogen is liberated quantitatively.

The time required for a determination varies from 45 minutes to 1.5 hours, depending upon the oxidizability of the hydrazine being determined.

This procedure, because of its specificity for hydrazines, is preferred to methods that do not differentiate between hydrazines and other nitrogen-containing compounds, such as the Kjeldahl and Dumas methods.

APPARATUS

The apparatus, shown in Figure 1, consists essentially of a reaction flask, *F*, in which the nitrogen is liberated, a Lunge nitrometer, *J*, in which the liberated nitrogen is measured over 50% potassium hydroxide, and a cylinder of purified carbon dioxide, *A*, which is used to displace the air from the apparatus prior to an analysis and to sweep the liberated nitrogen into the nitrometer.

The 100-ml. reaction flask, *F*, is attached to the apparatus by a $ 24/40 joint. The reagents are introduced through the separatory funnel, *G*, and the delivery tube, *L*, which has a maximum diameter of 3 or 4 mm. and a constriction at the bottom to prevent displacement of the liquid during decomposition of the sample. The reflux condenser, *H*, which is sealed to *M*, has an internal diameter of 12 mm. and allows vigorous refluxing of the reactants.

The carbon dioxide rate is controlled by the needle valve, *B*, and is estimated by the bubble counter, *E*, which is filled with an inert liquid such as butyl phthalate.

Other essential parts of the apparatus are the safety manometer, *C*, the leveling bulbs, *D* and *K*, and the three-way stopcock, *I*, which permits by-passing the nitrometer.

Commercial tank carbon dioxide is purified prior to use by venting rapidly about 50% of the carbon dioxide from the cylinder. A cylinder of carbon dioxide purified by this procedure contains a negligible impurity and contains sufficient carbon dioxide for several hundred analyses.

PROCEDURE

The apparatus is prepared for an analysis by completely displacing the air with carbon dioxide up to the reaction flask, *F*. The mercury in the safety tube, *C*, is lowered to a point slightly below the curved section of the manometer, so that carbon dioxide can be passed through the manometer to the atmosphere. After the air is completely displaced from the manometer, the leveling bulb, *D*, is raised until the mercury level is approximately halfway up the manometer. The carbon dioxide rate is then increased, and in several minutes the air will be completely displaced up to *F*. The delivery tube, *L*, is filled with water.

A sample that will give from 15 to 25 cc. of nitrogen is weighed into *F*, which is securely fastened to the apparatus by tension springs. Stopcock *I* is opened to the atmosphere, and carbon dioxide is rapidly passed through the apparatus until the air is completely displaced by carbon dioxide. This will acquire from 5 to 10 minutes. After displacing the air, the carbon dioxide rate is reduced to 1 to 2 bubbles per second. *I* is closed so the carbon dioxide passes into the nitrometer, and after several minutes microbubbles are obtained. The air collected in the nitrometer is displaced, and the leveling bottle lowered until it is about level with the nitrometer inlet tube. Forty milliliters of saturated copper sulfate, 15 ml. of 95% sulfuric acid, and 10 ml. of distilled water are drawn into the reaction flask. The solution is boiled until the reaction is complete and microbubbles are obtained. The carbon dioxide rate can be increased after the reaction appears complete to speed up sweeping the liberated nitrogen into the nitrometer. A blank is then determined on an equal volume of copper sulfate, sulfuric acid, and water and is usually about 0.6 ml.

Four different hydrazines representing two monosubstituted aryl hydrazines, a trisubstituted and a tetrasubstituted derivative, respectively, were prepared, purified, and subsequently subjected to analysis using the procedure outlined above. Nitrogen content was also determined by the conventional Dumas method.

Table I. Experimental Results

Hydrazine	Nitrogen Calcd. %	Nitrogen Found: Dumas method %	Nitrogen Found: This method %	Deviation: From calcd. N %	Deviation: From Dumas N %	Time Required for Analysis *Hours*
Phenylhydrazine hydrochloride	19.38	19.33	19.23	−0.15	−0.10	0.75
			19.32	−0.06	−0.01	
o-Tolylhydrazine hydrochloride	17.64	17.42	17.40	−0.24	−0.02	0.75
			17.32	−0.32	−0.10	
Benzaldehyde phenylhydrazone	14.27	14.14	14.15	−0.12	+0.01	1
			14.18	−0.09	+0.04	
2-Acetyl-1-benzoyl-1-phenyl hydrazine	11.02	10.90	10.82	−0.20	−0.08	1.5
			10.97	−0.05	+0.07	

Experimental results are presented in Table I. The two analytical procedures check well with each other, thus establishing the validity and usefulness of the method. Both procedures give results which are somewhat low compared with calculated values for nitrogen. The new method can be carried out rapidly to give results that compare favorably with the older Dumas procedure.

The better agreement between the experimental nitrogen values than between the experimental and calculated values can be attributed to the slight decomposition of the hydrazines when recrystallized from warm solution.

The volume of the blank, the vapor pressure of the potassium hydroxide, Charles' law, and Boyle's law were taken into consideration in the calculation of results.

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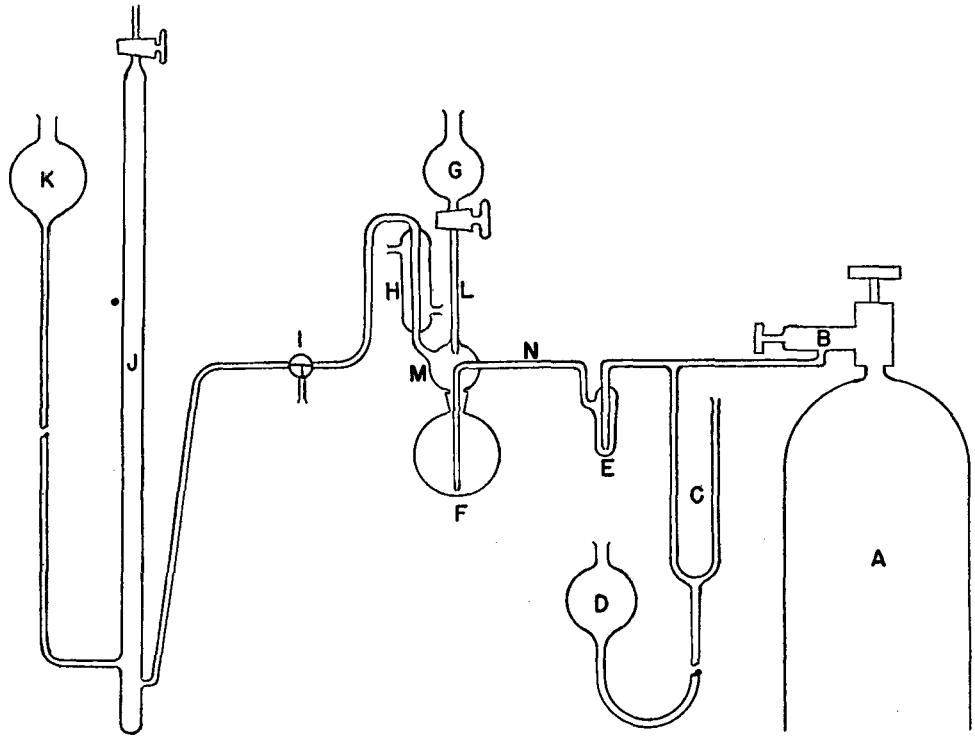

Figure 1. Diagram of Apparatus

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Separation of Antimonic Chloride from Antimonous Chloride by Extraction into Isopropyl Ether

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The distribution of pentavalent and trivalent antimony between hydrochloric acid solutions and isopropyl ether has been studied for various concentrations of acid and antimony. An extremely rapid equilibrium results in a quantitative extraction of pentavalent antimony over the acid range 6.5 to 8.5 *M* with a pseudo-distribution coefficient greater than 200. The distribution coefficient for the extraction of trivalent antimony is 0.016. A procedure using two distributions will quantitatively and rapidly separate pentavalent and trivalent antimony.

THE preferential extraction of antimonic over antimonous chloride by ethyl ether from hydrochloric acid solutions was reported in 1911 by Mylius and Huttner (*3*), whose studies were made over the acid range 5 to 20% (1 to 6 *M*). Under their most favorable conditions 85% of pentavalent antimony and 6% of trivalent antimony were extracted into the organic layer, as shown in Figure 1. Because of the inefficiency of the separation and the critical acid concentration required, their method has seen little use as a means of separation of the two valence states of antimony. For the similar extraction of ferric chloride from hydrochloric acid solutions isopropyl ether has been shown (by Dodson, Forney, and Swift, *1*) to possess certain advantages over ethyl ether. Consequently isopropyl ether has been studied as an extractant for the separation of antimonous and antimonic chlorides. The range of acid concentrations studied was extended beyond those covered by Mylius and Huttner. The use of isopropyl ether proved to give a rapid and very efficient separation of trivalent and pentavalent antimony over a wide range of hydrochloric acid concentrations.

EXPERIMENTAL METHOD

Distribution data were obtained on three types of hydrochloric acid solutions of the antimony chlorides. One type of solution contained only trivalent antimony, a second only pentavalent antimony, and the third contained equimolar mixtures of the two. In the studies reported here, the extraction was not tried over a range of small to large values of the ratio of trivalent to pentavalent antimony.

In one experiment one of these solutions was placed in a separatory funnel and isopropyl ether, saturated with hydrochloric acid (11.6 *M*), was added. The extraction was carried out by shaking the aqueous and organic phases for 5 minutes at room temperature. The separatory funnel was then allowed to stand until the two layers separated and the lower aqueous layer was drawn off. This aqueous layer was analyzed for either trivalent or pentavalent antimony. From the amount of trivalent or pentavalent antimony found remaining in the aqueous layer the amount extracted into the organic layer was calculated. For those solutions initially equimolar in trivalent and pentavalent antimony, duplicate extractions under identical conditions were then carried out in order to determine the amount of the other valence form extracted.

EXPERIMENTAL

The isopropyl ether used had a boiling range of less than 2° and gave a negative test for peroxide. Various solutions of antimonous and antimonic chloride were prepared using C.P. crystalline antimonous chloride and reagent grade antimonic chloride. Standardization of the trivalent solutions and all analyses for trivalent antimony were made using sulfatoceric acid as oxidant (*4*). Standardization of the pentavalent antimony solutions and subsequent analyses for pentavalent antimony were made by the potassium iodide method (*2*). Standard solutions required in the analytical procedures were prepared by conventional methods.

All the hydrochloric acid solutions of the antimony chlorides were 8 ml. in volume. In the extraction studies on solutions

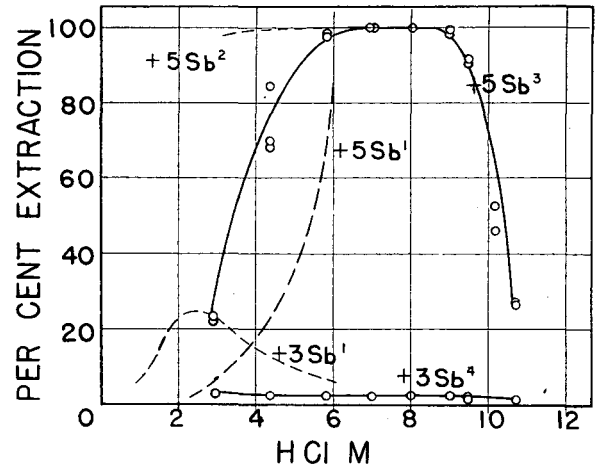

Figure 1. Extraction of Pentavalent and Trivalent Antimony from Hydrochloric Acid Solutions, Using Ether Extractants

1. Extraction of Sb^{+++++} and Sb^{+++} using ethyl ether (*3*)
2. Extraction of Sb^{+++++} from solutions containing only Sb^{+++++} using isopropyl ether
3. Extraction of Sb^{+++++} from solutions containing equimolar amounts of Sb^{+++++} and Sb^{+++} using isopropyl ether
4. Extraction of Sb^{+++} from solutions containing only Sb^{+++} or equimolar amounts of Sb^{+++} and Sb^{+++++} using isopropyl ether

equimolar in trivalent and pentavalent antimony, for example, the solutions were prepared by pipetting 1 ml. of the pentavalent stock solution and 1 ml. of the trivalent stock solution into a 50-ml. separatory funnel. These stock solutions contained 60 mg. per ml. of antimony dissolved in 11.6 *M* hydrochloric acid. Additions of 11.6 *M* acid and water were then made using semimicroburets to bring the initial concentration in hydrochloric acid to the desired value. The resulting solutions were thus 0.0625 *M* in each form of antimony. The initial acid concentration was calculated by assuming the 2 ml. of antimony stock solutions to be equivalent to 2 ml. of 11.6 *M* acid. The acid concentrations of the solutions containing antimony are purely formal rather than molar concentrations, as there is undoubted association of the antimony and acid in the aqueous phase. Although this is recog-

Table I. Distribution of Pentavalent and Trivalent Antimony between Hydrochloric Acid Solutions and Isopropyl Ether[a]

Initial HCl Concentration	Final Volume of Aqueous Layer	Extracted into Ether Layer	
		Sb^{+++++}	Sb^{+++}
Moles/liter	*Ml.*	%	%
2.9	7.8	23.5	4.0
2.9	..	23.4	3.0
2.9	..	23.5	..
4.35	7.9	69	2.3
4.35	..	84.5	2.4
4.35	..	70	2.3
5.8	7.9	97.7	2.3
5.8	..	98.2	2.4
6.96	7.9	100	2.3
6.96	..	100	2.45
8.0	..	100	2.3
8.0	..	100	2.3
8.0	..	100	2.4
9.0	..	99.8	2.3
9.44	9.4	91.7	2.3
9.44	..	91.4	2.0
10.15	10.2	53	..
10.15	..	46	..
10.58	..	27	1.0
10.58	..	27	1.7

[a] Original aqueous solutions contained 0.0625 *M* Sb^{+++} and 0.0625 *M* Sb^{+++++} and were 8 ml. in volume.

nized and appreciated, the term "molar" has been used to describe the acid concentration.

Twenty milliliters of isopropyl ether previously saturated with hydrochloric acid were then added. It is possible to use isopropyl ether without prior saturation with hydrochloric acid. In this case the volume change of the aqueous layer will be greater, particularly at higher acid concentrations, than if the ether is previously saturated. However, in either case the volume change is not appreciable.

The magnitude of the volume change using isopropyl ether previously saturated with acid is shown in Table I.

RESULTS

The results of the distribution experiments for systems equimolar in trivalent and pentavalent antimony are collected in Table I, and curves showing the percentage extractions at various acid concentrations for all three types of systems studied are shown in Figure 1. The results of Mylius and Huttner using ethyl ether are also shown in Figure 1.

An inspection of Table I and the curves of Figure 1 shows that the extraction of pentavalent antimony by isopropyl ether becomes more effective with increasing acid concentration up to about 6 *M*. There is a plateau from about 6 to 9 *M* for which the extraction of pentavalent antimony is virtually complete. Beyond this acid concentration the extent of the extraction falls off rapidly.

If only pentavalent antimony is present, the extraction is at least 98% from 3 to 9 *M* acid, as shown in Figure 1. If only trivalent antimony is present, its extraction follows the curve for trivalent antimony for the equimolar system of Figure 1.

An experiment, run to investigate the possibility of chemical reduction of pentavalent antimony during the extraction, involved checking the pentavalent antimony stock solution for trivalent antimony, running a blank determination, and then checking the aqueous layer for trivalent antimony following the extraction of a solution containing only pentavalent antimony. Reduction was observed to an extent of not more than 0.2% of the initial amount of pentavalent antimony.

Distribution coefficients for the extraction of trivalent antimony by isopropyl ether were determined over a fivefold range of antimony concentrations (Table II). The extractions were carried out from solutions 8 *M* in hydrochloric acid. The coefficient, the ratio of trivalent antimony in moles per liter in the ether layer to that in the aqueous layer, is seen to be a true constant having the value 0.016 over this range.

The distribution coefficient for the extraction of pentavalent antimony by isopropyl ether under conditions of maximum extraction would be difficult to determine, as the extraction is so complete. A pseudoconstant considering the slight amount of chemical reduction involved could be given as somewhat greater than 200. This pseudoconstant is obtained from the ratio of moles per liter of pentavalent antimony in the ether layer to the amount of the original pentavalent antimony (now present as trivalent antimony) in the aqueous layer. It was not possible to detect any pentavalent antimony actually present in the aqueous layer.

The time for equilibrium was shown to be of the order of seconds by determining the per cent extraction of pentavalent antimony using short mixing times (Table III). The solutions were identical with those of Table I in antimony concentrations and were 8 *M* in acid.

Table II. Distribution Coefficient for Extraction of Trivalent Antimony

Initial Concentration of Sb^{+++}	Distribution Coefficient $\frac{Sb^{+++}\text{ (Ether)}}{Sb^{+++}\text{ (Aqueous)}}$
Mole/liter	
0.0645	0.015
0.0645	0.015
0.1325	0.016
0.1325	0.017
0.1883	0.016
0.1883	0.016
0.2546	0.016
0.2546	0.017
0.3123	0.017
0.3123	0.016

From these results it follows that by using a two-step procedure pentavalent and trivalent antimony are separated quantitatively and rapidly. A single distribution concentrates the pentavalent antimony and about 2% of any trivalent antimony in the organic layer. A second distribution is then made between this organic layer and hydrochloric acid (6.5 to 8.5 *M*). The pentavalent antimony remains in the organic layer but the trivalent antimony redistributes between the two phases, leaving only on the order of 0.04% of the original trivalent antimony in the isopropyl ether layer.

Table III. Time of Equilibrium

Time of Shaking	Sb^{+++++} Extracted
Sec.	%
300	100
60	100
30	100
10	98

ACKNOWLEDGMENT

The authors are greatly indebted to Jacqueline Jones for preparing several of the standard solutions used and carrying out certain of the distribution coefficient experiments.

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Self-Absorption of S^{35} Radiation in Barium Sulfate

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A technique has been developed for the collection of radioactive precipitates utilizing centrifuge tubes with removable flat bottoms. A study of the self-absorption of S^{35} activity in barium sulfate has been reported. A value of 0.216 sq. cm. per mg. was found for the self-absorption coefficient of S^{35} in barium sulfate on a Lusteroid backing.

A FREQUENTLY used method for studying a given type of radiation is to determine an absorption curve by measuring the intensity of transmitted radiation as a function of absorber thickness. In the case of a disintegration which produces simple β-spectra, such as that of P^{32}, C^{14}, or S^{35}, the intensity is nearly $I = I_0 e^{-\mu d}$, where d is the absorber thickness, and μ is the absorption coefficient. Actually the semilogarithmic plot of the observed intensity *vs.* the absorber thickness is generally not quite straight and μ is the average value of the slope in a suitable region of the graph.

For the ordinary absorption experiment, foils of various thicknesses are interposed between a fixed radioactive sample and the detector. If, on the other hand, the absorber consists of a carrier material which is mixed with the radioisotope, self-absorption results. A self-absorption plot is obtained by measuring the apparent activity of a series of samples which contain equal amounts of the radioisotope but different amounts of carrier. This graph may be fitted by the curve $I = I_0 \frac{1 - e^{-\alpha d}}{\alpha d}$ by proper choice of the self-absorption coefficient, α. Generally, the fit is better than might be expected, for the derivation of the formula requires exact exponential absorption and parallel tracks for the β-rays, and back-scattering from the mounting is ignored. This effect of the backing appears as a deviation from the theoretical curve in the region of small thickness.

A technique is described below for measuring S^{35} activity precipitated as barium sulfate using a thin mica window Geiger-Müller counter. The data obtained by this method give a value of $\alpha = 0.216$ sq. cm. per mg. for the self-absorption coefficient.

PROCEDURE

Aliquots of a standardized sample of sodium sulfate containing S^{35} were precipitated as barium sulfate in the flat-bottomed centrifuge tubes illustrated in Figure 1. The Lusteroid planchet which fomred the bottoms of these tubes were molded in a glycerol bath heated to 200° C. Inert sodium sulfate was added in varying amounts to permit the precipitate to range from 1 to 50 mg. per sq. cm. The aliquots containing inert carrier were diluted to approximately 50 ml. and acidified to pH 3 with hydrochloric acid, and then 10 ml. of a 10% solution of barium chloride were added dropwise with stirring. After addition of the barium chloride, the precipitate was allowed to age and then centrifuged at 2000 r.p.m. for 10 to 15 minutes. The supernatant was siphoned from the tubes, care being taken not to disturb the precipitate. The precipitate was resuspended in a small volume of distilled water, the sides of the tube were carefully washed, and the volume was made up to approximately 50 ml. The precipitate was again centrifuged and the clear supernatant removed. After again resuspending the precipitate, sufficient ethanol was added to the third wash to make a 50% solution. The samples were then centrifuged and the clear supernatant removed. The Lusteroid planchet was removed and the small volume of alcohol which could not be siphoned from the tube without disturbing the surface of the precipitate was allowed to evaporate at room temperature. Samples were finally dried in a vacuum desiccator over calcium sulfate and weighed.

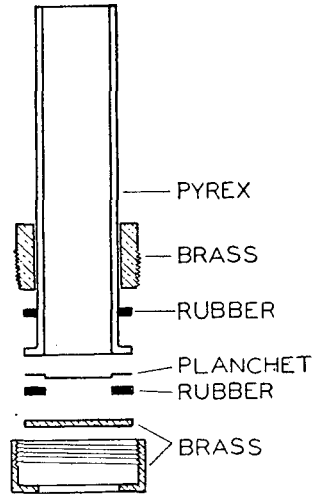

Figure 1. Collection Apparatus for Barium Sulfate Precipitate

The accuracy of the procedure as a method for gravimetric analysis for sulfate was demonstrated by analyses of a standard sample of sodium sulfate from which theory demanded a yield of 127.2 mg. of barium sulfate. The result of a series of seven analyses was 126.8 ± 0.44 mg. which was 99.7% of the theoretical yield.

RESULTS

The sample of S^{35} active barium sulfate was mounted over an area of 6.42 sq. cm. in the center of a plastic film planchet 3.8 cm. in diameter and 0.05 cm. thick. The sample was placed 4 mm. below a thin (2.3 to 2.7 mg. per sq. cm.) mica window counter with a diameter of 27 mm. The counting rates have been corrected for resolution, background, and, when necessary, for decay. Approximately 5000 counts were recorded for each sample. The data are summarized in Figure 2. Each point represents the average of two or three observations of activity at that sample thickness. The average value of each series of observed activity was plotted as the percentage of total activity and the curve has been drawn to fit the equation:

$$\text{Observed activity in per cent} = 100\left(\frac{1 - e^{-\alpha d}}{\alpha d}\right)$$

with $\alpha = 0.216$ sq. cm. per mg.

DISCUSSION

The data are well fitted by a curve of the type $I = I_0\left(\frac{1 - e^{-\alpha d}}{\alpha d}\right)$ where I is the observed activity, I_0 the activity without carrier, d the thickness of the sample in milligrams per square centimeter, and α the absorption coefficient. This equation has been used by

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Henriques (*4*), Libby (*7*), Solomon (*8*), and Yankwich (*9*). Parameters I_0 and α were chosen in order to obtain a good fit to the data in the working range of sample thickness from 1 to 50 mg. per sq. cm. The value of α obtained was 0.216 sq. cm. per mg.

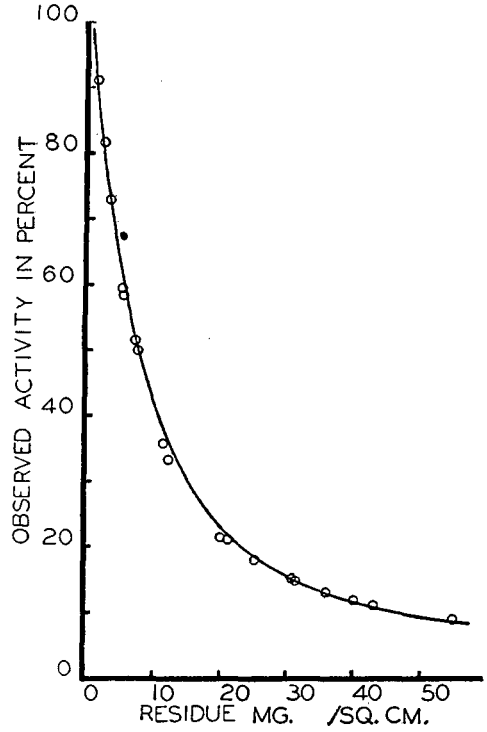

Figure 2. Geiger Counter Self-Absorption Curve for S^{35} in Barium Sulfate

Hendricks *et al.* (*3*) precipitated barium sulfate on brass disks and obtained a self-absorption curve. No theoretical curve was fitted originally but, using their value for the sample area, the data given are well fitted above 4.6 mg. per sq. cm. by using $\alpha = 0.28$ sq. cm. per mg. Below 4.6 mg. per sq. cm. the experimental curve falls below the theoretical and the extrapolated activity for 0 thickness is 87% of the theoretical.

Henriques *et al.* (*4*) employing benzidine sulfate precipitation onto filter paper obtained a value of $\alpha = 0.265$ sq. cm. per mg., fitting the region from 1 to 11 mg. per sq. cm.

Yankwich (*10*) and Glendenin (*2*) have found that the theoretical absorption curve fits the experimental data for sample thicknesses larger than 20% of the range, while for thinner samples a departure is observed due to the difference in back-scattering from the planchet material as compared to the carrier material. On this basis one would expect better agreement with the values given by Hendricks *et al.* and the authors. The value obtained by Henriques for the 1 to 11 mg. per sq. cm. range is definitely a function of the backing.

The discrepancies noted here are not consistent with the theory in its present state. For accurate work, a self-absorption curve should be run by the experimenter himself.

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Rapid Qualitative Method for Acrylonitrile

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Acrylonitrile can be identified by conversion to β-piperidinopropionitrile, the picrate of which is a suitable derivative. Water and organic solvents, except the stronger acids and bases, do not interfere. Acrylonitrile in concentrations as low as 1% can be detected and identified by this method. Methyl and ethyl acrylate can be similarly characterized by conversion to the picrates of the methyl and ethyl esters, respectively, of β-piperidinopropionic acid.

IN CONNECTION with its use in nitrile rubbers the need arose for a rapid and simple method of identifying acrylonitrile. No such method has been described in the literature. A great variety of crystalline derivatives obtained by the cyanoethylation reaction have been described, but the procedures involved do not in general lend themselves to adaptation as simple qualitative methods. Among the most rapid reactions of acrylonitrile are those with certain nonaromatic amines. The aminonitriles obtained are themselves liquids, but may be readily converted into solid derivatives. Such a method for characterizing acrylonitrile has the advantage of greater speed and convenience than the usual methods for nitriles, and water and various other substances do not interfere.

AMINO DERIVATIVES OF ACRYLONITRILE

Piperidine and morpholine (*6*), certain substituted piperidines (*1*), and some of the primary aliphatic amines (*3*) react vigorously with acrylonitrile in the absence of catalyst to give the corresponding β-aminopropionitriles. The present study was confined to the use of morpholine and piperidine, particularly the latter. The properties of the two aminonitriles and their derivatives are listed in Table I.

Table I. Properties of Derivatives of Acrylonitrile

	Beta-Substituted Propionitrile	
	Piperidino	Morpholino
Boiling point, ° C.	233/740 mm. 129–130/30 mm. (*6*) 105/11 mm.	149/20 mm. (*6*) 128/9 mm.
Melting point, ° C.	−7.4 to −7.2	20.4 to 20.6
n_D^{20}	1.4692; 1.4697 (*6*); 1.4713 (*4*)	1.4709; 1.4710 (*6*)
	Melting Point, ° C.	
Picrate	161–162; 160 (*6*) 157.5–158.0 (*4*)	138–140; 139.5 (*6*)
Methiodide	158; 152 (*2*)	193–195 with decomp.
Hydrochloride	181–182	210–211
Picrate of parent secondary amine	150–151	148–150

Of the derivatives listed, the picrates are much the simplest to prepare. Addition of either aminonitrile to a saturated solution of picric acid in ethanol results in an immediate precipitate of the picrate. After recrystallization from ethanol, the picrates have reproducible melting points. The piperidino derivative is preferred because its picrate has a higher melting point than that of the parent secondary amine, whereas the reverse is true of morpholine. Hence, there is less chance for uncertainty with piperidine if in case of a negative test for acrylonitrile the picrate of the secondary amine happens to be obtained. However, morpholine has been successfully used to characterize samples of acrylonitrile.

REAGENTS

Piperidine. Eastman Kodak Company white label grade or other material of similar quality is satisfactory.

Picric Acid Solution. Excess reagent grade picric acid (containing 10 to 12% of water) is shaken with commercial denatured ethanol and the solution saturated at room temperature is decanted from the undissolved solid.

PROCEDURE FOR QUALITATIVE TEST

For an unknown which is suspected to consist chiefly of acrylonitrile, the recommended procedure is as follows:

A small volume (0.1 to 1.0 ml.) of unknown is treated with a somewhat less than equal volume of piperidine. The mixture is allowed to stand 2 minutes and then added to 5 to 20 ml. of picric acid solution. The resulting precipitate is recrystallized once from ethanol, filtered, and washed with a little ethanol. The product is dried for a few minutes on the suction funnel and the melting point is determined. If the melting point falls in the range of about 161° to 162° C., this will serve to identify the sample as acrylonitrile.

Notes. Heat is evolved when piperidine is added to acrylonitrile. A little spontaneous boiling may occur, but with small samples cooling is not necessary. Heat is also evolved when piperidine is added to various other compounds, notably acids and certain aldehydes. However, if the sample of unknown is reasonably pure, failure to observe a temperature rise virtually constitutes a negative test. Dilute solutions of acrylonitrile in inert solvents may not give a noticeable temperature rise when treated with piperidine.

When the piperidine-acrylonitrile reaction mixture is added to picric acid solution, the picrate comes down immediately as an intense yellow powder or as flat yellow platelets. The product is only sparingly soluble in boiling ethanol. Recrystallization can be accomplished quickly by simply leaching the solid for 1 to 2 minutes with about 20 ml. of boiling ethanol, filtering by gravity while the mixture is still hot, and chilling the filtrate to recover the purified product. By this procedure picrates melting at 161–162° C. were obtained. The melting points were not altered by further recrystallization.

From the data in Table III it is apparent that the method can be used to detect relatively small amounts of acrylonitrile in a variety of mixtures.

For such application the mixture should first be freed of acid or strong base by any appropriate neutralization, extraction, or distillation procedure. A sample of 0.5 to 5.0 ml. of the neutral mixture is treated with 0.10 ml. of piperidine and the mixture is allowed to stand 10 minutes before being added to about 5.0 ml. of picric acid solution. If no precipitate is obtained at once, the mixture should be allowed to stand a few minutes.

An alternative procedure, useful for detecting as little as 1% acrylonitrile in a solvent more volatile than β-piperidinopropionitrile, is as follows:

To 20 ml. or more of solution is added about 0.20 ml. of piperidine, and the mixture is refluxed or heated to 100° for 10 minutes. Solvent is then removed by distillation until the distillation residue has been reduced in volume to 2 to 3 ml. A few drops of the cooled residue are added to picric acid solution and the precipitate is treated as above.

EXPERIMENTAL

The effect of varying the ratio of piperidine to acrylonitrile is illustrated in Table II. Samples of 0.10 ml. of acrylonitrile were treated with various volumes of piperidine, and the mixtures were allowed to stand 30 seconds and then poured into 5.0 ml. of picric acid solution.

Table II. Effect of Amount of Piperidine

(0.10 ml. of acrylonitrile. 5 ml. of picric acid solution)

Vol. of Piperidine, Ml.	Effect
0.025	Immediate turbidity, voluminous precipitate after 5 minutes
0.050	Immediate dense precipitate
0.10	Immediate dense precipitate
0.20	Immediate slight precipitate
0.25	No precipitate
0.50	No precipitate

The effect of excess piperidine and of other amines was further illustrated by a series of tests in which a mixture of 0.10 ml. each of acrylonitrile and piperidine was added to 5 ml. of picric acid solution, and the slurry of picrate in mother liquor was treated with just sufficient amine to redissolve the precipitate. The following volumes were required: piperidine, 0.10 ml.; morpholine, 0.15 ml.; tri-*n*-butylamine, 0.20 ml.; aniline, no effect with 5.0 ml.

Table III. Effect of Solvents

(Acrylonitrile, 0.10 ml. Piperidine, 0.10 ml. Picric acid, 5.0 ml.)

Solvent	Volume, Ml.	M.p. of Precipitate ° C
Water[a]	10.0	159–161
Ethanol	10.0	+[b]
n-Butanol	10.0	158–160
n-Hexane	2.0	159–162
n-Hexane	10.0	−[b]
Light mineral oil	1.0	+
Ethyl ether	1.0	+
Isopropyl acetate	1.0	+
Isopropyl acetate	2.0	−
Ethyl chloride	1.0	158–160
Ethyl chloride	2.0	−
Acetonitrile	1.0	+
Propionitrile	1.0	158–160
Acrylonitrile[c]	5.0	160–161
Methacrylonitrile	1.0	160–162
Methacrylonitrile	2.0	−
Acetone	0.4	161–162
Acetone	1.0	157–158
Methyl ethyl ketone	1.0	+
Methyl ethyl ketone	2.0	−
Methyl isobutyl ketone	1.0	159–160
Methyl isobutyl ketone	2.0	−
Formalin, 37%	0.4	158–160
Formalin, 37%	1.0	+
Acetic acid	1.0	−
Benzene	1.0	+
Toluene	1.0	+
Toluene	2.0	−
Styrene	1.0	161–162
o-Dichlorobenzene	1.0	+
Nitrobenzene	1.0	160–161
Nitrobenzene	2.0	+
Aniline	1.0	159–161

[a] Picric acid solution was diluted with 5.0 ml. of ethanol to avoid precipitating picric acid.

[b] A plus sign indicates that a picrate was obtained but its melting point was not determined. A minus sign indicates that no picrate precipitated.

[c] Included to show that a large excess of acrylonitrile does not interfere.

The effect of solvents was studied by adding 0.10 ml. of acrylonitrile to the solvent, then adding 0.10 ml. of piperidine, and after 5 minutes treating the mixture with 5.0 ml. of picric acid solution. The results are summarized in Table III. The melting points are those of the once recrystallized picrates.

DISCUSSION

If the sample of unknown is neutral or nearly so and contains a significant amount of acrylonitrile, the most likely cause of failure of the method is the use of too high a proportion of piperidine. This is illustrated by the data of Table II. The aminonitrile is formed but its picrate is not precipitated in the presence of excess piperidine. Other bases of the same order of basic strength interfere for the same reason, but aniline is too weak a base to cause interference.

Acids such as acetic acid apparently interfere by conversion of the piperidine to the corresponding nonreactive salt.

With inert solvents interference probably arises through retarding of the reaction between acrylonitrile and piperidine by simple dilution effect. For example, if the mixture of 0.10 ml. of acrylonitrile, 1.0 ml. of benzene, and 0.10 ml. of benzene (Table III) is allowed to stand for 10 minutes instead of 5 before being added to picric acid solution, a considerably larger amount of picrate is obtained. Acrylonitrile at 1% by volume in benzene is readily detected by adding the appropriate amount of piperidine, refluxing a few minutes, then distilling off most of the benzene before adding the mixture to picric acid solution. At room temperature the reaction appears to be faster in water and alcohols than in other solvents.

Of special interest is the noninterference of methacrylonitrile, which reacts very slowly with piperidine. No heat is evolved when one volume of piperidine is added to two of methacrylonitrile. After 24 hours, a 0.30-ml. sample of the mixture gives no precipitate when added to 5.0 ml. of picric acid solution. After standing 5 days the mixture gives a picrate melting at 140–141° C.

IDENTIFICATION OF ACRYLATES

The above procedure has been successfully used to characterize methyl and ethyl acrylates. The picrates of the methyl and ethyl esters of β-piperidinopropionic acid so obtained melt at 106–108° C., with sintering at 102° C. and 127.5–128.0° C. (*5*), respectively.

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Estimation of Acetic Acid as an Impurity in Refined Formic Acid

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Concentrations of acetic acid (0 to 6%) in refined formic acid have been determined quantitatively by oxidation of the formic acid with excess mercuric oxide and titration of the remaining acetic acid with standard alkali to a preselected pH. Rapid routine analyses have been accomplished by this technique, which employs ordinary laboratory apparatus and reagents.

IN THE recovery and refining of formic acid from a crude stock containing both formic and acetic acids, it was necessary from a control standpoint to have a rapid, quantitative method for estimating acetic acid in the refined product. A method was required which would be more nearly quantitative than the standard specification test (*1*) for acetic in formic acid. This test is semiquantitative and indicates only whether the acetic acid content is "less than 0.4%." A method was desired for determining acetic acid quantitatively in amounts as low as 0.1% in 85 to 90% formic acid. Partition and azeotropic distillation methods (*2*, *4*, *5*) were not applicable because of the unfavorable ratio of formic to acetic acid and the time-consuming features of the latter technique.

A method has been developed for the estimation of acetic acid in formic acid samples in which acetic acid is known to be the only acidic impurity present. The method is based on the oxidation of formic acid to carbon dioxide and water by an excess of mercuric oxide, and potentiometric titration of the remaining acetic acid with standard alkali. The interference of mineral acids and organic salts may be prevented, where necessary, by the application of precipitation or distillation procedures before applying this test. When present, the higher homologs of the acetic acid series will interfere, because their acidity will be determined with that of the acetic acid.

PROCEDURE

Preacidify samples known to contain less than 1% acetic acid with 10 ml. of a standard solution containing 5 ml. of glacial acetic acid in 1 liter of water. Add a similar 10-ml. portion to the blank. Use 5 grams of sample if the acetic acid content is below 2%, or 2 grams if above 2%. For a practical titration, the weight of acetic acid present in the reaction should be between 50 and 150 mg.

To a 300-ml. Erlenmeyer flask add the sample, 10 ml. of the standard acetic acid solution (if necessary), enough mercuric oxide (Mallinckrodt's A.R., yellow) to provide 5.5 grams present for each gram of formic acid in the sample, and enough water to make a total volume of about 30 ml. Prepare a blank, containing the same quantities of reagents but no formic acid, and carry it along simultaneously with the sample. Connect the flask to an efficient water-cooled reflux condenser and heat the contents of the flask slowly for about 10 minutes. During the early stages of the reaction, carbon dioxide is evolved vigorously. Following the initial reaction period, apply sufficient heat to provide a positive reflux which is continued for 90 minutes.

At the end of the reflux period, add 20 to 25 ml. of water through the condenser to provide rinsing and dilution. All samples at this point must show the presence of excess mercuric

oxide. When cooled to room temperature transfer the contents of the flask quantitatively, without filtration, with 20 to 25 ml. of water to a 250-ml. beaker and titrate potentiometrically with 0.1 *N* sodium hydroxide solution (1 ml. is equivalent to 0.006 gram of acetic acid). Although the volumes of water used in the procedure are not critical, they are believed to be near optimum and permit simplification and standardization.

Very vigorous stirring is necessary during the titration, and a magnetic stirrer, with a polythene-covered stirring bar, is recommended. When the procedure as outlined above is followed, the required end point is obtained at pH 8.6 for the titration of the sample, and at pH 8.3 for the titration of the blank which contains no formic acid. The difference in titer between the blank and the sample is equivalent to the acetic acid present in the sample. Samples requiring a titration volume of more than 10 to 15 ml. of 0.1 *N* sodium hydroxide may not give a sharp end point and an appreciable period of vigorous stirring is necessary before a stable final pH is obtained.

$$\% \ HC_2H_3O_2 = \frac{\text{ml. net titer} \times N \ NaOH \times 0.060}{\text{weight of sample (grams)}} \times 100$$

DISCUSSION

The reaction between mercuric oxide and the lower members of the fatty acid series may be represented by the following equations. A normal degree of thermal stability is shown by all these salts except the one obtained from formic acid (*1*, *3*).

$$HgO + 2HCOOH \longrightarrow Hg(OOCH)_2 + H_2O$$

$$2Hg(OOCH)_2 \xrightarrow{(heat)} 2HgOOCH + CO_2 + HCOOH$$

$$2HgOOCH \xrightarrow{(heat)} 2Hg + CO_2 + HCOOH$$

In the presence of excess mercuric oxide, the net reaction becomes

$$HgO + HCOOH \xrightarrow{(heat)} Hg + CO_2 + H_2O$$

Although this series of reactions proceeds rapidly at temperatures near 100° C., an appreciable reaction time is required to ensure total destruction of the formic acid. Under these same conditions, acetic acid will form a mercury acetate or will remain as free acid if present in high dilution. Potentiometric titration of this acid, both free and in its mercury salts, may be made using standard sodium hydroxide solution. Following the destruction of the formic acid by this method, a portion of any acetic acid which is present will be found to exist as the mercurous salt. The only effect of this side reaction—which is negligible in samples containing less than 75 mg. of acetic acid—is to increase the time required to attain a stable end point during titration.

In the preliminary investigation of the method, it was observed that the presence of acetic acid greatly increased the rate of decomposition of the mercury salts of formic acid. When samples of laboratory purified (distilled) formic acid were tested for acetic acid, reaction periods as long as 16 hours were necessary to cause the apparent acetic acid content to approach a stable minimum value. When a small measured quantity of acetic acid was added to the samples, the apparent acetic acid reached a stable minimum value with a reaction time of 1 hour. For this reason, samples of formic acid which are believed to contain less than 1% acetic acid should be preacidified by the addition of a small measured quantity of acetic acid. By this technique, a satisfactory breakdown of the formic acid can be accomplished in a practical span of time, regardless of the acetic acid concentration of the sample. This preacidification also serves to expel carbon dioxide from the reaction mixture.

Colorimetric indicators are not entirely satisfactory for use during the titration without prior removal of the excess mercuric oxide. Experiment has shown that satisfactory results can be obtained by titration to a stable, preselected pH, as ascertained by a conventional pH meter. Except under unusual conditions the slope of the neutralization curve is satisfactorily sharp in the vicinity of the end point. Titrations are most easily accomplished using 0.1 *N* sodium hydroxide solution.

An investigation was made of the effect of large quantities of mercuric oxide and metallic mercury on the potentiometric titration of small quantities of acetic acid. Although the general shape of the neutralization curve is altered slightly, the equivalence point remains practically the same as that obtained for acetic acid alone under comparable conditions of dilution.

The mechanical or aspiration loss of organic acidity from a reaction mixture of formic acid, acetic acid, mercuric oxide, and water was found to be negligible when the period of vigorous initial decomposition was controlled.

Table I. Acetic Acid Recovery from Formic Acid–Acetic Acid Blends

% Acetic Acid			No. of
Added	Found	Recovered	Detns.
0.00	0.09	..	6
0.25	0.33	97	6
0.50	0.56	95	8
1.83	1.88	98	5
5.2	5.20	98	6

EXPERIMENTAL DATA

Mercuric oxide from various sources was tested for interference. Of the five samples examined, none was found that was not suitable, provided it was of the yellow variety and of reagent grade. Tests were made to show the effect of these different mercuric oxides on a water solution containing approximately 25 mg. of acetic acid and on 5-ml. samples of formic acid containing 0.50% acetic acid. In the former case acetic acid recovery ranged between 99.8 and 101.4%. In the latter case the acetic acid content was found to be between 0.52 and 0.59%.

A practical evaluation of the method was made by testing a series of prepared blends of formic and acetic acids. The formic acid used was laboratory distilled material collected on the anhydrous formic acid plateau. This material was diluted to approximately 90% formic acid by the addition of water. Grasselli glacial acetic acid was used as the other component. For simplification of the test, all blends were prepared as weight-per-volume acetic acid in formic acid and the final results were calculated on this basis. The results of these tests are listed in Table I. The values reported under Recovered have been corrected for the 0.09% acetic acid found in the formic acid used. The acetic acid content of this material, as determined by mass spectrometer analysis, was 0.07%.

Seven samples of commercial refined formic acid, 87 to 90%, from five different supply houses, were analyzed for acetic acid and found to contain between 0.04 and 0.15%. In duplicate determinations the average deviation was 0.014% with a maximum deviation between determinations of 0.029%.

PRECISION AND ACCURACY

A precision of approximately ±0.03% and an accuracy within 0.1% absolute are possible when the method is applied to 90% formic acid containing 0 to 6% acetic acid.

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Spectrochemical Determination of Hafnium-Zirconium Ratios

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A spectrochemical technique is presented for determining hafnium-zirconium ratios in the range 100 Hf/Zr = 0.073 to 9.28 by weight, with and without the aid of hafnium-free zirconium preparations. The sample is brought into solution in 10% sulfuric acid and sparked by the porous cup technique. The intensity ratio Hf II 2641.406/Zr 2761.911 is measured, and the hafnium-zirconium ratio is read from a standard curve.

THE determination of hafnium in zirconiferous materials by traditional analytical methods has always been a singularly difficult task. No colorimetric reagent is known which is specific for one of these elements in the presence of the other. Those methods based on the effect of the hafnium-zirconium ratio on various gravimetric conversion factors rapidly lose accuracy at extreme values of the ratio because of the relatively large effect of errors in weighing and other chemical operations. Those based on selective precipitation of either constituent are liable to relatively large errors caused by coprecipitation.

In addition to these difficulties, it has heretofore been almost impossible to obtain zirconium preparations which were completely free of hafnium. Consequently, any comparison of mixtures with "pure" zirconium rested on an insecure foundation.

As an analytical technique, x-ray emission spectrography (*13*) enjoys certain inherent advantages over the methods mentioned. The analytical data are produced specifically by the inner electrons of hafnium, and are not affected by the presence of zirconium. This, in turn, enables one to dispense with a pure zirconium matrix. As a result, much of the analytical work on hafnium-zirconium mixtures has been performed by this method. X-ray emission analysis is rather time-consuming, however, and the necessary apparatus is not commonly available.

The success of the x-ray emission method and the comparative unavailability of pure zirconium and hafnium preparations are probably responsible for the almost total lack of published material on the determination of hafnium-zirconium ratios by optical emission spectrochemical methods. The few papers which mention the subject (*7*, *10*) are semiquantitative in treatment, and give no working curves or detailed analytical procedures.

Spectrochemical methods for the construction of working curves usually require the use of standards which resemble the sample in gross composition, and which contain known amounts of the element to be determined. It is not necessary for this purpose, however, that compounds of either the matrix element (zirconium, in this case) or the element to be determined (hafnium) be available in the pure state. If the concentration of hafnium in the hafnium preparation is known, its concentration in the zirconium preparation can be determined.

Two spectrochemical procedures for estimating the concentration of residual impurities have been described by Gatterer (*2*). Although these methods make no assumptions as to the shape of the working curve, they involve a fair amount of calculation. It was therefore decided to adopt the empirical, but reliable, method of trial additions.

This method is based on the assumption that a plot of log intensity *vs.* log (true) concentration is always a straight line. No exceptions to this have been found so far in the writer's experience. The method is in common use and has been described by Pierce and Nachtrieb (*8*).

First a working curve plot is made of log intensity ratio *vs.* log apparent concentration. When a residuum is present, the line will tend to become parallel to the concentration axis at low concentrations. A straight line is drawn through the points for the higher concentrations, and the amount of the residuum is estimated by the magnitude of the departure of the lower concentration points from this line. Next, all concentrations are corrected by adding the estimated residual value, and the process is repeated. Usually, two or three approximations will suffice to give a value which will bring all points onto a straight line. Finally, the correctness of the estimation is tested by using the working curve so obtained to determine spectrographically the amount of residuum in the pure matrix material.

Before this procedure can be used, however, the precision and accuracy of the data must be sufficient to give a rather narrow range of residuum concentration values which will bring all points onto a straight line. The original points must be rather close to the best smooth curve drawn through them, so that it will be possible to judge accurately when the best "straight line" has been achieved.

Several factors will combine, in this case, to affect the precision and accuracy of the results obtained:

Accuracy of Standards. The composition of the hafnium preparation mixed into the zirconium matrix must be accurately known. In this analysis, we are confronted by the fact that hafnium compounds free of zirconium are almost as difficult to obtain as are their opposite numbers. For instance, the first sample of hafnium oxide used in the present investigation was stated by the supplier to contain less than 1% zirconium. Spectrochemical analysis showed it to contain 25.0% zirconium as the oxide. This condition will, of course, be harmless if known about and corrected for in the calculation of standard compositions when the curve is plotted.

Background Correction. The success of the above-mentioned procedure for constructing a working curve with the use of impure matrix material depends largely on the accuracy and precision of the intensity ratios obtained for the lower concentration points. The use of an accurate background correction is therefore a matter of major importance. In view of this fact, it was desired to have an intensity scale which was as accurate as possible at low intensities.

The flatness of the density–log intensity and density-intensity curves at low intensity values tends to make the calibration marks on an intensity scale rather inaccurate in this region.

Prefogging the film is sufficient in itself to enable one to avoid working in the flattest regions of the density curves, but it shortens the useful range of intensities if used to the extent necessary to reach the linear portion of the H and D curve. If an intensity scale accurate in the lower intensities is available, however, a slight prefogging is useful in drawing attention to weak backgrounds for which corrections might not otherwise be made.

The advisability of prefogging is illustrated by the following hypothetical situation, which is typical of those encountered in spectrochemical photometry:

A line having an intensity of 1.5 (arbitrary) units is situated in a region having a background intensity of 0.3 unit. A spectrogram of this region is developed without prefogging; the intensity of the line (plus background) is registered and measured as 1.8, but the intensity of the adjacent background appears to be zero, due to the inertia of the emulsion. Thus, no background correction is made, and the intensity obtained for the line is in error by 20%. However, if the emulsion is prefogged sufficiently to give a "clear plate" intensity reading of 1.0, the line (plus background) will give an intensity reading of 2.8, and the spectrum background will read 1.3. Subtraction will then give the correct value for the line intensity.

A suitable film calibration function was found in the Seidel function, W, which is defined for a given line (or background) reading by the equation

$$W = \log\left(\frac{T_0}{T} - 1\right)$$

where T_0 is the "clear plate" galvanometer deflection and T is the deflection for the background or line (plus background) under consideration.

This function has been discussed by Kaiser (*5*) and by Honerjäger-Sohm and Kaiser (*4*).

With SA1 film, at 2600 Å., the Seidel function gives an almost perfectly straight line between 4 and 97% transmission when plotted against log intensity. The slope of the line is moderate, usually lying between 0.9 and 1.8. If the ordinates are made proportional to W, but the ordinate scale is calibrated in terms of T/T_0, per cent transmittance values and relative intensity values can be interpolated from this curve rather accurately over a wide range. This property substantially increases the accuracy of the low intensity calibrations.

Conditions in the Light Source. A stable light source is one of the main prerequisites for obtaining precise and accurate intensity ratios. The difficulty of controlling arc conditions where zirconium and hafnium are concerned makes it advisable to convert samples and standards to some uniform chemical state for analysis. Even when this is done, however, zirconium is notoriously sensitive to variations in arc conditions, as is evident from eloquent complaints registered against this element by Strock and Drexler (*11, 12*).

Any attempt to volatilize hafnium in an arc, with or without zirconium, appeared certain to involve high—and sometimes difficultly controllable—arc temperatures.

When it became necessary for this laboratory to determine hafnium-zirconium ratios spectrochemically, it therefore seemed advisable to use a spark-solution method. The porous cup technique (*1*), in which a solution slowly seeps through the bottom of a graphite cup used as the upper electrode, seemed well suited for this analysis. In addition to being able to handle the rather viscous and highly acid solutions likely to be encountered, this technique offered the possibility of using uniformly controlled spark excitation, and of having the composition of the radiating vapor vary in a reproducible manner, if at all, during the exposure.

EXPERIMENTAL

Preparation of Stock and Sample Solutions. Stock solutions of zirconium and hafnium were prepared as follows:

The appropriate oxide was fused with potassium pyrosulfate, and the melt was dissolved in approximately 200 ml. of hot 1% sulfuric acid. The hydroxide was then precipitated with alkali. In order to reduce buffering action as much as possible, the neutralization was performed with 10% sodium hydroxide solution until a precipitate began to form; the precipitation was then continued with ammonium hydroxide. The precipitate was allowed to settle, the bulk of the supernatant solution decanted, and the remaining slurry centrifuged. The supernatant solution was again decanted, and the residue washed twice with water containing a few drops of ammonia. The hydroxide was next dissolved in the smallest possible volume of 1 to 1 sulfuric acid and the solution was diluted with 10% sulfuric acid until it contained approximately 1% of the metal.

The metallic concentration of stock solutions was determined by assaying with cupferron. (The hydroxide could not be quantitatively precipitated with ammonia because of the complexing action of the large amounts of sulfuric acid present, *6*). Stock solutions were then diluted to the desired strength with 10% sulfuric acid.

Purity of Hafnium Oxide. A preliminary qualitative spectrographic analysis of the purest hafnium available at the time failed to show any impurities other than minor amounts of zirconium and traces of silicon and titanium. (This material was obtained from the Chemical Commerce Corp., Newark 8, N. J.) Chemical determinations carried out by F. J. Miller showed the two latter elements to be present in concentrations of about 0.15 and 0.1% as the oxides, respectively.

The zirconium content of this hafnium oxide was determined as follows:

A solution of this material containing approximately 1 mg. per ml. of hafnium was prepared and assayed as above. The proper amount of 1% thorium nitrate solution (calculated as the metal) was added to make the solution 200 p.p.m. in thorium.

A spectrogram of this solution was then obtained by the porous cup method, and the intensity ratio Zr II 3273.047/Th 3324.754 was measured. The zirconium concentration of the solution was read from a standard curve, and the zirconium-hafnium ratio calculated to be 0.022 by weight. (Control experiments had shown the presence of 1 mg. per ml. of hafnium to have no measurable effect on this intensity ratio.) The zirconium content of this material was, of course, taken into account in calculating standard concentrations.

Choice of Optimum Working Conditions. CHOICE OF LINES. In view of the abundance of zirconium lines, it was decided that a minor zirconium line could be used as an intensity reference line. This was a definite advantage, because it eliminated both the need for knowing the exact concentration of the sample solution and the need for adding an internal standard element.

Hafnium Lines. To find the hafnium lines showing the greatest sensitivity by the porous cup technique, a spectrogram was obtained of a solution containing 25 p.p.m. of hafnium as the metal in 10% sulfuric acid. Of the nine hafnium lines visible at that concentration between 2300 and 4300 Å., those listed in Table I are the most prominent.

Table I. Most Sensitive Lines of Hafnium

Wave Length		Possible Interferences
Hf II 2641.406	C II 2641.44	Was not detected in exposures of Zr solutions free of Hf
	Th 2641.49	Would not interfere under these experimental conditions unless sample contained ~5% Th
	Fe 2641.65	Found to be too weak to interfere in ore sample preparations studied so far
Hf II 2773.357	None pertinent	Proximity of several Zr and Hf lines makes accurate background correction difficult
Hf II 2820.224	Th 2820.337	Would not interfere under these experimental conditions unless sample contained ~20% Th. Proximity of Zr II 2818.739 makes accurate background correction somewhat difficult

The material dealt with was believed to be essentially free of thorium. It appeared unlikely that C II 2641.44 would cause trouble, and because Hf II 2641.406 was approximately 25% more intense than Hf II 2820.224, and had no close neighbors, it was the principal line used.

Zirconium Lines. In order to cover the maximum range of hafnium concentrations with the least variation in sample concentration, it was desirable that the zirconium line selected be in the middle density range under standard operating conditions. Zr 2761.911 suited the requirements best; Zr II 2583.405, although somewhat too intense to give maximum accuracy over the

entire range of sample concentrations, appeared satisfactory for use with the more dilute solutions.

It was suggested by Gatterer and Junkes (*3*) that the sum of two line ratios be used as an analytical dependent variable in order to even out inequalities in excitation. In accordance with this suggestion, and in view of the characteristics of the lines mentioned, the three following ratios were selected for further investigation:

A. $\frac{\text{Hf II } 2641.406}{\text{Zr } 2761.911}$

B. $\frac{\text{Hf II } 2641.406 + \text{Hf II } 2820.224}{\text{Zr } 2761.911}$

C. $\frac{\text{Hf II } 2641.406 + \text{Hf II } 2820.224}{\text{Zr } 2761.911 + \text{Zr } 2583.405}$

Choice of Sample Concentration. It was desirable to know to what extent, if any, a given hafnium-zirconium intensity ratio varied with the concentration of the sample. This was important in two connections:

It determined whether the concentration level of a given solution could be adjusted to suit its hafnium content without affecting the hafnium-zirconium intensity ratio obtained. (It would naturally be desirable to vary the sample concentration inversely with the expected hafnium concentration in order to keep the hafnium line at a moderate density level.)

It determined to what extent it was necessary to control the (Zr + Hf) concentration of a sample solution. Such control, if necessary, would involve gravimetic or colorimetric assaying, and would be very time-consuming.

The effect of sample concentration was therefore investigated as follows:

A zirconium preparation in which the hafnium-zirconium ratio was ~1.9% by weight—i.e., 100 Hf/Zr ~1.9—was made up in three dilutions covering the range Zr = 2.3 to 9.4 mg. per ml. Four samples of each solution were sparked for 180 seconds apiece, and the above-mentioned ratios were measured at each concentration.

The results of this set of measurements, given in Table II, do not show any clear-cut variation of a given ratio with sample concentration. It therefore appeared permissible to increase or decrease the sample concentration within this range if hafnium lines proved too light or too dense, respectively, for accurate photometry. Unless there was reason to expect an extremely high or extremely low hafnium-zirconium ratio, solutions were therefore made up to a concentration of approximately 5 mg. of zirconium per ml.

Because the results of this study were intended to apply to routine determinations, it was felt that the superior performance of ratios B and C was not worth the additional time necessary to measure the additional lines. Attention was therefore concentrated on ratio A.

Choice of Length of Exposure. It was desired to use as long an exposure as possible, in order to bring out weak hafnium lines, if present. The longest exposure period normally obtainable with a single filling of the porous cup is 180 seconds. Experience with this technique has shown that it is safest to use a single uniform exposure period when dealing with some concentrated solutions; all exposures were therefore made for 180 seconds.

Table II. Effect of Sample Concentration

Intensity Ratio	Value of Intensity Ratio at Indicated Zr Concn.		
	2.3 mg./ml.	4.7 mg./ml.	9.4 mg./ml.
A	0.980 ± 0.06	1.02 ± 0.03	0.930 ± 0.05
B	1.75 ± 0.070	1.81 ± 0.10	1.65 ± 0.05
C	0.639 ± 0.020	0.659 ± 0.020	0.639 ± 0.015

Exposure, Development, and Photometry. All exposures were made on an ARL-Dietert 1.5-meter grating instrument having a dispersion of 7 Å. per mm., using SA1 film. The slit-width was 20 microns. Excitation was provided by a Baird high voltage spark source.

Films were prefogged by a 5-second exposure to the light of a 25-watt Mazda lamp operated at 40 volts, situated 90 cm. (3 feet) from the film. A 7.5-cm. (3-inch) section of film not in the region of interest was kept covered during the prefogging.

The films were next developed 3 minutes in Du Pont x-ray developer at 18° C., and fixed for 3 minutes in Eastman F-5 fixer.

The film was then sandwiched between two thin glass plates and mounted on the plateholder of a Leeds & Northrup microphotometer. A "clear plate" reading was taken on an unexposed portion of the unfogged section of the film, and photometer tracings were made of Hf II 2641.406 and Zr 2761.911 and the adjacent regions. The intensities of these lines and their respective backgrounds were measured on the tracing with an intensity scale prepared for 2600 Å. using the Seidel function. (The necessary calibration was performed on the same roll of film as was used in the present investigation.) The background intensity was subtracted from the (line plus background) intensity, and the appropriate ratio was calculated.

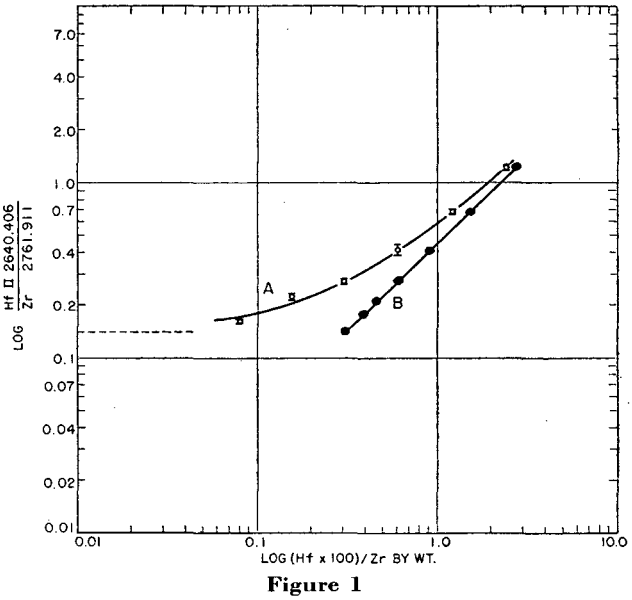

Figure 1

Construction of Working Curve. WITH IMPURE BASE MATERIAL. A sample of zirconium oxide obtained from the Foote Mineral Company, Paoli, Pa., was used as base material.

A stock solution containing 5.12 mg. per ml. of zirconium, calculated as the metal, was prepared as described above. A 2-ml. aliquot of this material (aliquot A) was taken, and 50 micrograms of a 0.498% solution of the above-mentioned hafnium were added to it.

The stock zirconium solution was then diluted to 0.976 times its original strength to compensate for the decrease in the zirconium concentration in aliquot A caused by addition of the hafnium solution. Five successive 1 + 1 dilutions of aliquot A were then made with the diluted stock zirconium solution. These six solutions, plus a sample of the stock zirconium solution, were exposed in quadruplicate as described above, and the intensity ratio Hf II 2641.406/Zr 2761.911 (ratio A) was measured.

The hafnium-zirconium concentration ratios and the observed intensity ratios, corrected for background, are given in Table III. In the left-hand column, r represents the residual amount of hafnium present in the zirconium-base material.

Curve A in Figure 1 shows the log of these intensity ratios plotted against the log of the normal 100 Hf/Zr ratios—i.e., ratios in which r is assumed to be equal to zero. The log of the intensity ratio for 100 Hf/Zr = r is shown as a horizontal dotted line for curve A, as the abscissa corresponding to $r = 0$ is $-\infty$.

Table III. Hafnium-Zirconium Ratios

$\frac{Hf \times 100}{Zr}$, by Weight	Intensity Ratio, $\frac{Hf\ II\ 2641.406}{Zr\ 2761.911}$
$r + 2.43$	1.20 ± 0.03
$r + 1.22$	0.67 ± 0.02
$r + 0.607$	0.41 ± 0.03
$r + 0.304$	0.27 ± 0.01
$r + 0.152$	0.22 ± 0.01
$r + 0.076$	0.16 ± 0.00
r	0.14 ± 0.01

A trial value for r was selected by inspection, and added to concentration values selected at evenly spaced intervals along the smooth curve, A. The curve was then replotted, using the log of the new concentration as abscissa, and the log of the intensity ratio shown by A for the nominal concentration in question as ordinate. B is the curve obtained in this manner for $r = 0.31$. Lowest point on B is that for the solution in which 100 Hf/Zr = r.

The accuracy of the value of r as determined by this method is estimated as $\pm \sim 0.01$, as the lines obtained for $r = 0.29$ and $r = 0.33$ showed definite positive and negative curvature, respectively.

As a method of constructing working curves, however, this procedure has the weakness that the hafnium concentration range lying below the hafnium content of the base material must be reached by extrapolating the corrected curve, if it is to be reached at all.

WITH PURE BASE MATERIAL. Several months ago, this laboratory was fortunate enough to obtain a sample of pure zirconium oxide prepared at the National Bureau of Standards under the direction of E. Wichers. This material was estimated by B. F. Scribner to contain less than 200 p.p.m. of hafnium. (No hafnium was detected in it spectrographically, either by Scribner or the author.)

Table IV. Intensity Ratios

$\frac{Hf \times 100}{Zr}$, by Weight	Intensity Ratio, $\frac{Hf\ II\ 2641.406}{Zr\ 2761.911}$
$r + 9.28$	4.05 ± 0.35
$r + 4.64$	1.95 ± 0.16
$r + 2.32$	0.98 ± 0.007
$r + 1.16$	0.504 ± 0.020
$r + 0.580$	0.242 ± 0.017
$r + 0.290$	0.145 ± 0.008
$r + 0.145$	0.065 ± 0.009
$r + 0.073$	0.043 ± 0.002
r	...

As this afforded an opportunity to check the accuracy of the previous curve, a stock solution was prepared from this material. A known amount of hafnium was added to an aliquot of this stock, and successive dilutions were made as above with the stock solution. The hafnium-zirconium ratio in each of these solutions is given in Table IV. Inasmuch as the zirconium base material may have contained as much as 200 p.p.m. of residual hafnium, r may be different from zero, but is no greater than 0.02%.

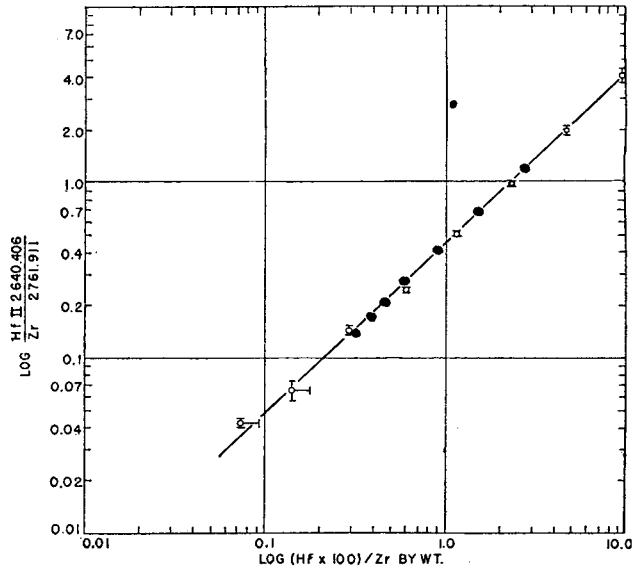

Figure 2

Owing to the high intensity of the hafnium lines resulting from the two highest concentrations, it was necessary to dilute these solutions. They were diluted to approximately 1.3 mg. of zirconium per ml. with 10% sulfuric acid. The resulting weakening of the zirconium line probably decreased the accuracy of the photometry in these cases, but lack of material prevented repetition of the exposures. The intensity ratios obtained are given in Table IV and plotted in Figure 2. The solid circles on this curve are the points used to plot curve B of Figure 1. The possible residuum of hafnium in the National Bureau of Standards zirconium oxide has been neglected in plotting all but the two lowest concentrations. In the latter two cases, its possible presence is indicated by concentration limit marks placed at log 0.093 and log 0.165, respectively.

Curve B of Figure 1 agrees with the curve obtained with the National Bureau of Standards material to well within the limits of experimental error over the concentration range common to both.

ACCURACY. In order to check the accuracy of analytical figures obtained by the present method, three zirconium preparations were circulated among the laboratories listed in Table V. Three leaders in the table indicate that the sample was not sent to the

Table V. Comparison of 100 Hf/Zr Ratios (by Weight) Obtained by Various Methods

Analyst	Method	Sample No. 1	Sample No. 2	Sample No. 3
		Result	Result	Result
C. Feldman[a]	Spectrochemical (porous cup)	1.94 ± 0.04	0.31 ± 0.01	0.25 ± 0.02
B. F. Scribner[b]	Spectrochemical (d.c. arc)	...	0.42	...
V. A. Fassel[c]	Spectrochemical (pellet, over-damped spark)	...	0.31	...
H. S. Pomerance[a]	Capture cross section for thermal neutrons	2.0 ± 0.2[d]	...	...
S. A. Reynolds and G. C. Bell[a]	Activation analysis (neutron-induced 45-55-day Hf activity)	2.0 ± 0.2	0.29 ± 0.03	...
C. O. Muehlhause[e]	Activation analysis (neutron-induced 19-second Hf activity)	4.3	...	0.27 ± 0.01

[a] Oak Ridge National Laboratory, Oak Ridge, Tenn.
[b] National Bureau of Standards, Washington, D. C.
[c] U. S. Atomic Energy Commission Laboratory, Iowa State College, Ames, Iowa.
[d] Based on values of $(0.30 \pm 0.07) \times 10^{-24}$ and $(103 \pm 3) \times 10^{-24}$ sq. cm. for cross sections of Zr and Hf, respectively. The first figure is the mean of a value obtained by appropriately weighting cross sections obtained for separated zirconium isotopes and a value obtained on the pure base material (*9*). The second figure was obtained with pure natural HfO_2.
[e] Argonne National Laboratory, Chicago, Ill.

laboratory in question. An attempt was made to cross-check several independent methods. Unfortunately, it was impossible to obtain an x-ray emission analysis in time for publication.

Comparison of these results shows agreement to within limits of experimental error in almost all cases. No checks have yet been made at higher hafnium concentrations.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Amanda Estepp and John Gillespie, who prepared the solutions and obtained the spectrograms reported here. Mr. Gillespie also obtained some of the photometric data.

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Determination of Calcium in Presence of Nickel and Cobalt

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A method is described whereby a mixture of the chlorides of calcium, nickel, and cobalt is analyzed without a preliminary separation of the metals with hydrogen sulfide. Ammonium oxalate is used to precipitate the calcium from an ammoniacal solution. The precipitate is titrated with standard potassium permanganate, and nickel in the filtrate is determined with dimethylglyoxime. Cobalt is calculated by difference from a total chloride determination on a separate sample. The accuracy of the method was established by test determinations on several synthetic mixtures of widely varying composition.

DURING work on the extraction of inorganic salts from aqueous solution (*2*), it became necessary to analyze mixtures of the chlorides of calcium, nickel, and cobalt. In order to avoid the inconvenient conventional precipitation of the nickel and cobalt with hydrogen sulfide and the subsequent dissolution of the sulfides preparatory to the separation of these metals, attention was directed toward the development of a procedure in which calcium is removed from nickel and cobalt by direct precipitation as calcium oxalate.

REAGENTS

The mixtures of salts to be analyzed were made up with C.P. analyzed grade materials. Compounds used in the analytical procedure were all of reagent grade.

PROCEDURE

The sample is introduced into a 125-ml. Erlenmeyer flask and diluted to a volume of about 50 ml. Enough concentrated ammonia is added to convert the nickel and cobalt to the ammine complexes (a marked color change in the cobalt present characterizes this point). The ammonia is added fairly rapidly to avoid more than the momentary appearance of the intermediate insoluble basic salts. (These salts have a tendency, if allowed to form and come out of solution for more than a very brief period, to dissolve with extreme slowness in excess ammonia.) To the clear ammoniacal solution is added enough 4% ammonium oxalate to precipitate the calcium and provide a slight excess. The flask is stoppered and allowed to stand overnight. The mixture is filtered through a Gooch crucible and the precipitate washed with water. The calcium oxalate and asbestos mat are taken up with 50 ml. of dilute sulfuric acid (1 volume of acid to 10 volumes of water) and titrated at 90° C. to a pink end point with standard 0.1 *N* potassium permanganate (prepared according to Booth and Damerell, *1*.)

The filtrate from the above determination is neutralized (litmus paper test) with 1 to 1 hydrochloric acid and nickel is determined by the standard dimethylglyoxime method (*3*, *5*).

The cobalt content is calculated by difference from a total chloride determination carried out on a separate sample by the standard gravimetric silver nitrate method.

DISCUSSION

The procedure described is an extension and modification of a method for calcium and nickel reported by Kuhl (*4*). He separated calcium as the oxalate from an ammoniacal solution, ignited the residue, and weighed the resulting calcium oxide. Nickel was determined gravimetrically as nickel dimethylglyoxime. There was no indication of the possible effect of cobalt. Furthermore,

Table I. Analysis of Synthetic Mixtures of Calcium, Nickel, and Cobalt Chlorides

Mixture	$CaCl_2$, Mg.			$NiCl_2$, Mg.			$CoCl_2$, Mg.		
	Present	Found	Error	Present	Found	Error	Present	Found	Error
1	14.2	14.5	+0.3	21.4	21.7	+0.3	27.5	27.2	−0.3
2	42.6	42.4	−0.2	13.4	13.5	+0.1	27.5	27.9	+0.4
3	56.8	56.6	−0.2	5.3	5.4	+0.1	55.0	55.4	+0.4
4	63.9	64.3	+0.4	13.4	13.3	−0.1	5.5	5.6	−0.1
5	8.5	8.8	+0.3	0.5	0.6	+0.1	22.0	21.9	−0.1
	Av. error, mg.		±0.3			±0.1			±0.3

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this method called for the addition of ammonium chloride, eliminating the use of a chloride balance for cobalt.

Preliminary qualitative work showed that both nickel and cobalt are slowly precipitated from neutral solutions by ammonium oxalate. These precipitates dissolve upon the addition of ammonia. Such ammoniacal solutions remain clear when exposed to the atmosphere for one week. After this period, some nickel salt separates from solution, presumably through the loss of ammonia, for the precipitate redissolves upon the addition of more ammonia to the solution.

The procedure was checked by employing it for the analysis of five synthetic mixtures of calcium, nickel, and cobalt chlorides in varying proportions, prepared from stock solutions of pure salts. The results are presented in Table I, which indicates the method to be reliable and free from systematic error. The average error for each component borders on the limit of accuracy of the analytical technique used for its determination in the stock solutions.

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Colorimetric Determination of Serum Iron

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Iron is determined colorimetrically as the complex formed with thiocyanate. To increase the specific color intensity of the solution to be measured, the iron complex is extracted and concentrated in ether. The ether extract is measured at 500 mμ, where its absorption is greatest. For the analysis of serum iron, nitric acid is used to precipitate proteins, to split off iron bound to proteins, and to oxidize ferrous iron. No digestion is necessary. Although the method is designed for determination of serum iron, it may be used for the estimation of small amounts of iron in other solutions.

MANY investigators including Wong (*10*), Moore *et al.* (*8*), Barkan (*1*), and several others (*2–4, 6, 7, 9*) have developed colorimetric methods for the determination of serum iron, utilizing the colored complex formed in the reaction of ferric iron with thiocyanate.

In the Wong method, organic iron is released by oxidation with sulfuric acid and potassium persulfate. The proteins are precipitated with tungstic acid. Thiocyanate is added to the filtrate and the iron is determined colorimetrically. The low extinction coefficient for small amounts of $FeSCN^{++}$ in aqueous solution and the relatively high reagent blank found in this procedure make serum iron determinations resulting from this method subject to rather large errors.

In the Moore method the serum is digested with sulfuric acid and hydrogen peroxide. The oxidized iron is then treated with thiocyanate and the iron complex thus formed is extracted with isoamyl alcohol. The higher extinction coefficient of the isoamyl alcohol solution of the iron complex improves the accuracy of the determination over the Wong method. However, the technique requires large amounts of serum (5 to 10 ml.) and the acid digestion procedure is time-consuming.

In the Barkan method, which can use as little as 1.5 ml. of serum, "peroxide" ether is used as a solvent for the thiocyanate color. The use of the unstable "peroxide" promises certain disadvantages in routine use in many laboratories.

In the investigation described here an attempt was made to develop an accurate and rapid method for the estimation of small amounts of serum iron. Nitric acid is used to precipitate serum proteins, to split off protein-bound iron, and to oxidize any ferrous iron present. To the ferric iron thus made available potassium thiocyanate is added. The iron complex is then extracted with ether and concentrated before being measured colorimetrically.

PROCEDURE

Clear, hemoglobin-free serum (1.5 ml.) is diluted with 4.5 ml. of distilled water and acidified with 6 ml. of 1 *M* nitric acid. The mixture is allowed to stand for at least 5 minutes for complete precipitation, is then centrifuged and the precipitate is discarded. An 8-ml. aliquot of the clear supernatant is poured into a 60-ml. separatory funnel and 1.6 ml. of 3 *N* potassium thiocyanate are added. The mixture is shaken for 1 minute with about 3 volumes of peroxide-free ether which has been saturated with water, and the ether layer is separated. This extraction is repeated twice, and the three ether fractions are combined, evaporated to a small volume, and made up quantitatively to 5 ml. with ether. Because

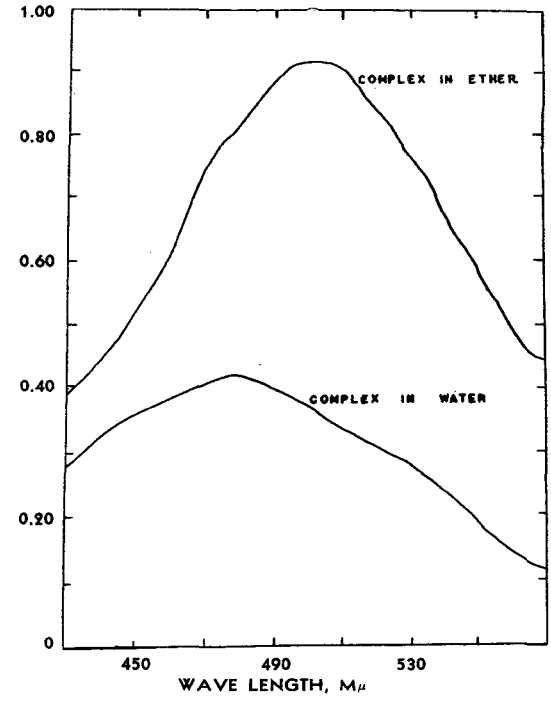

Figure 1. Spectral Absorption of Iron Thiocyanate Complex in Ether and in Water

of the easy volatilization of the ether, any convenient vessel may be used for the concentration; care should be taken to avoid loss by entrainment. Final adjustment of the volume may be made in a tube graduated to the precision required. For many purposes a 10-ml. graduated pipet or even a graduated centrifuge tube is satisfactory.

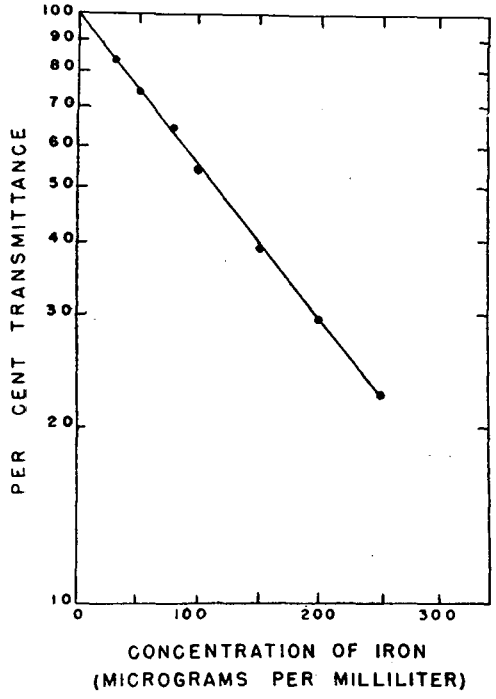

Figure 2. Extinction Coefficients of Standard Samples

The iron concentration is then determined by comparing the color intensity with that of a standard iron solution treated in the same way. In the transfer and handling of the ether solution after adjustment to volume, care must be taken to avoid large losses of the highly volatile solvent. Cuvettes used for measuring should be stoppered.

EXPERIMENTAL

Measurements for this study were made with a Beckman D.U. spectrophotometer at 500 mμ, using the blue-sensitive photocell and a slit width of 0.04 mm. However, with a filter of the proper spectral characteristics (such as the Wratten No. 75 filter) any colorimeter may be used. No normally occurring constituent of serum other than iron furnishes an ether-extractable compound in the procedure described which has an absorption band in the measured range. Use of the method should not be limited to determination of serum iron but should be applicable to other solutions containing small amounts of iron.

The serum iron in ten samples of normal serum was determined by both the method of Moore and the method outlined here. The results listed in Table I show good agreement between the two methods.

Recovery experiments were run in which iron was added to five analyzed samples of serum. In this set of analysis, 1 ml. of a standard iron solution (100 micrograms per 100 ml.) was added to 1 ml. of the serum and this mixture was then analyzed. The results in Table II show quantitative recovery.

As shown in Figure 1, the iron complex in ether saturated with water gives a maximum absorption at 500 mμ as compared to 475 mμ for aqueous solutions. Figure 1 also shows that the specific absorption of the iron complex in the ether solution is greater. Several organic solvents, such as isoamyl alcohol (as used by Moore *et al.*). isopropyl alcohol, petroleum ether, and chloroform were tested for the extraction of thiocyanate iron complex. Of those tested, ether gave the most complete extraction and the highest absorption for the iron complex. The intensity of the iron complex color was found to be stable for several hours.

To check the linearity of the color reactions, the extinction coefficients of standard samples of varying concentrations were determined. That Beer's law applies over a useful concentration range for serum iron is shown in Figure 2.

Table I. Comparison of Nitric Acid–Ether Extraction Method and Moore Method for Determination of Serum Iron

Sample	Nitric Acid–Ether Extraction Method	Moore Method	Δ
	γ of iron in 100 ml.		
1	87.2	85.5	+1.7
2	84.6	85.6	−1.0
3	86.8	87.3	−0.5
4	98.4	97.8	+0.6
5	102.3	99.6	+2.7
6	88.6	89.8	−1.2
7	89.7	88.6	+1.1
8	91.6	90.0	+1.6
9	107.8	106.0	+1.8
10	89.9	89.3	+0.6

To determine the completeness of the extraction of the iron complex with ether, the partition coefficient for the iron thiocyanate complex between ether and water was experimentally determined and found to be 8.09 at 28° C. Three ether extractions reduced the iron in the water residue to a negligible amount. When the iron concentration is low, the large volume of comparatively dilute ether solution thus obtained may necessitate evaporation to concentrate the iron complex for more accurate analysis. No change was found in the final iron values of analyzed samples which were read before evaporation and after evaporation.

As in any method for the determination of serum iron, this procedure is based on the use of iron-free reagents and hemoglobin-free serum. When hemoglobin is present in the serum, a correction may be made by measuring the absorption at 576 mμ and calculating the hemoglobin iron. (Hemoglobin is 0.33% iron, *5*).

Table II. Recovery of Known Quantity of Iron Added to Serum

	Serum Fe (Original)	Serum Fe plus Added Fe		Δ
		Theoretical	Experimental	
	γ of iron in 100 ml.			
1	88.6	188.6	190.6	−2.0
2	89.7	189.7	190.4	−0.7
3	91.6	191.6	187.4	+3.2
4	107.8	207.8	207.0	+0.8
5	89.9	189.9	188.4	+1.5

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Determination of Creatine and Creatinine in Urine

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An improved method for the determination of creatine and creatinine in urine using the Jaffe reaction is described. Accurate results for the determination of creatinine depend upon carefully controlling the hydrogen ion concentration of the reaction mixture, the temperature, and the heating time. The authors conclude that many of the disputes regarding the optimum conditions for the determination of creatine in urine may be resolved in the light of the very marked effect of hydrogen ion concentration.

THE literature contains many conflicting reports regarding the optimum conditions necessary for the determination of creatine and creatinine. The present study discusses the various factors that must be controlled in order to obtain accurate, reproducible determinations of creatine and creatinine in urine. The methods described have given very satisfactory results over a period of 2 years in the analysis of over 600 urine specimens.

EXPERIMENTAL

Instruments. The instruments used were the Beckman spectrophotometer, Model DU, Beckman pH meter, and the Evelyn colorimeter (Rubicon Company).

REAGENTS

Picric Acid Solution. Picric acid (C.P.) is recrystallized twice from glacial acetic acid and dried at room temperature, following the suggestion of Benedict (*2*). A 1.17% solution is used, following the recommendation of Peters (*11*).

Sodium Picrate Buffer. One liter of the 1.17% picric acid solution is adjusted to pH 2.0 ± 0.05 by the addition of approximately 20 ml. of 2 *N* sodium hydroxide. It is advisable to check the pH of the solution after several hours, because the freshly prepared solution tends to drift in pH.

Sodium Hydroxide Solution. One hundred grams of C.P. sodium hydroxide are dissolved in 1 liter of water and stored in a Pyrex bottle.

STANDARD

Creatinine Standard. Creatinine (Pfanstiehl) is recrystallized from water and dried over phosphorus pentoxide at 80° C. in vacuo.

ANALYSIS OF CREATININE ($C_4H_7N_3O$) USED. Calculated, C 42.47, H 6.24, N 37.15%. Found, C 42.16, H 6.28, N 37.14%.

A stock solution containing 1 gram per liter of 0.1 *N* hydrochloric acid is diluted 1 to 10 with water to give a standard of 0.1 mg. per ml.

Creatine Standard. Creatine (Pfanstiehl) is recrystallized from water and dried over phosphorus pentoxide at 80° C. in vacuo.

ANALYSIS OF CREATINE ($C_4H_9N_3O_2$) USED. Calculated, C 36.63, H 6.92, N 32.05%. Found, C 36.60, H 7.05, N 31.82%.

A stock solution containing 1 gram per liter of water is diluted 1 to 10 with water to give a working standard of 0.1 mg. per ml. All standards are maintained at 4° C. using an overlayer of toluene as a preservative.

CREATININE DETERMINATION

Procedure. Into a 125 × 25 mm. Pyrex test tube are pipetted 1 ml. of the creatinine standard, 4 ml. of sodium picrate buffer, and 0.3 ml. of sodium hydroxide solution. The solution is allowed to stand 20 minutes, diluted with 15 ml. of water, and mixed thoroughly, and the optical density is measured at a suitable wave length. The color is stable for several hours. Urine samples are usually diluted 1 to 10 before pipetting. A reagent blank, substituting water for the creatinine standard, is carried through each batch of determinations. It is desirable to run duplicate determinations on all samples, and triplicate determinations on the standard.

For the routine procedure, optical measurements are made in 1-cm. Corex cells with the Beckman spectrophotometer at 510 mμ wave length, using a slit width of 0.05 mm. After a preliminary reading of the reagent blank, the instrument is set to 100% transmittance against the blank. Measurements in the Evelyn colorimeter are made with a No. 520 filter.

Comparison of Results Obtained with Evelyn Colorimeter and Beckman Spectrophotometer. Simplification of both calculation and laboratory manipulation is possible if conditions can be controlled so that a colorimetric procedure follows the Beer-Lambert law. Figure 1 illustrates the dependency of the creatinine measurement upon the optical conditions of measurement. The wide band of the filter-type colorimeter apparently causes wide deviations from linearity between creatinine concentration and optical density, particularly with higher creatinine values. Greater sensitivity is obtained at 510 mμ than at 520 mμ

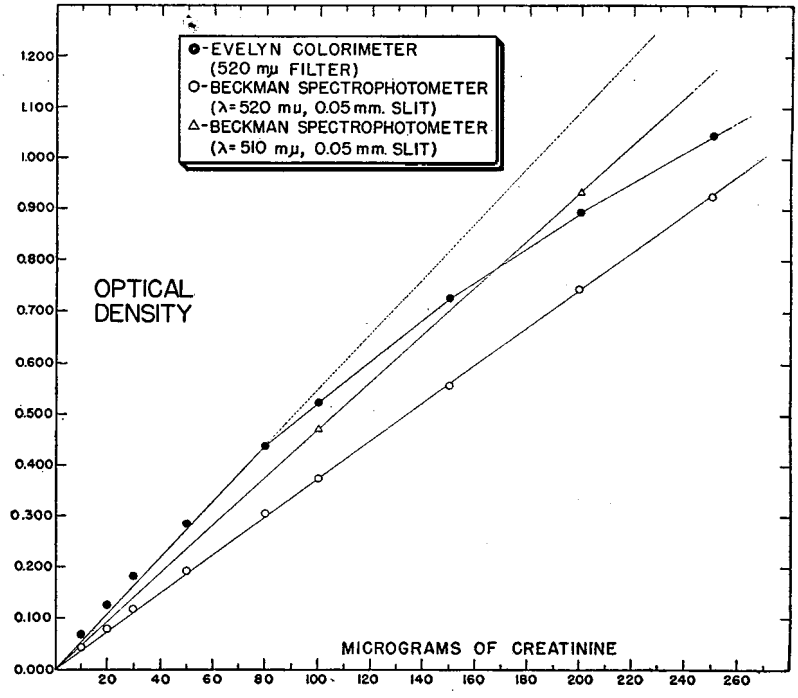

Figure 1. Comparison of Evelyn Colorimeter and Beckman Spectrophotometer in Determination of Creatinine

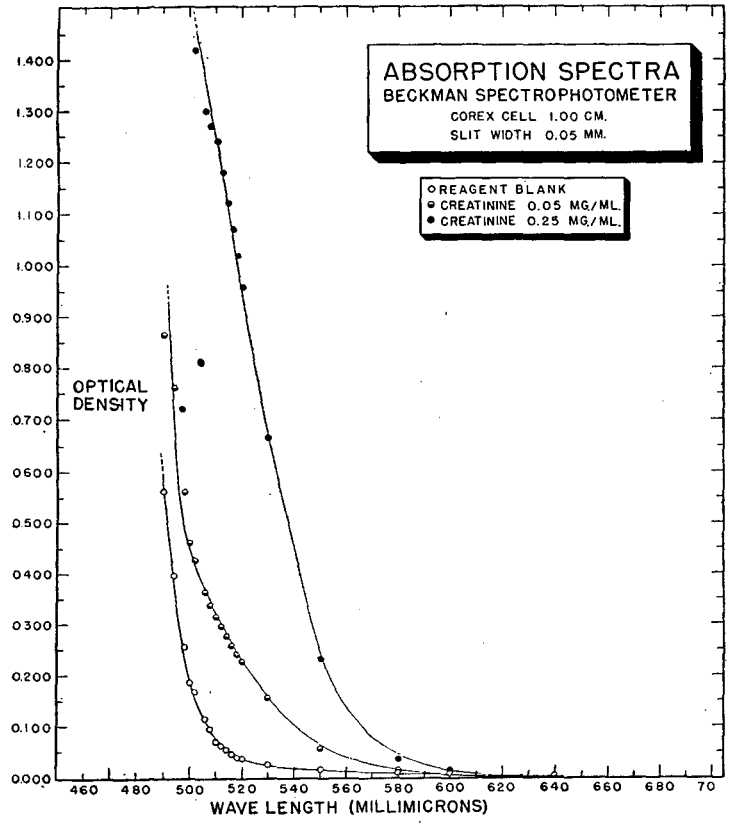

Figure 2. Spectral Absorption Curve

using the Beckman instrument. In order to obviate the construction of calibration curves, which usually must be checked frequently, the Beckman spectrophotometer has been used for all measurements described below.

Absorption Spectra. In Figure 2 are shown the absorption spectra obtained with the reagent blank and two different concentrations of creatinine. The curves illustrate that it is necessary to measure the colored reaction product using a very narrow wave band because of the strong absorption of the reagent itself at only a slightly shorter wave length.

Interfering Substances. Various drugs, glucose, acetone, acetoacetic acid, etc., may affect the reaction. All urines analyzed in this laboratory by this method are tested qualitatively for acetone and glucose. If either of these abnormal substances is present it should be eliminated.

CREATINE DETERMINATION

Procedure. The procedure for creatine measurement is identical to that used for creatinine except that the samples are heated, under controlled conditions, between the additions of sodium picrate buffer and sodium hydroxide. Triplicate creatine standards are analyzed with each batch of samples.

For routine procedure, the samples are heated for 30 minutes at 120° C. in an autoclave. Because evaporation is negligible under the conditions used here, it is not necessary to stopper the tubes, although they may be capped with metal foil to prevent entrance of foreign material. It is advisable to cool the samples immediately after autoclaving. The experimental findings which form the basis for this procedure are presented below.

Rate of Reaction. Both pH and temperature govern the rate of conversion of creatine to creatinine in the autoclave, and under the conditions of these experiments a negligible destruction of creatinine occurs.

Effect of pH. Figure 3 indicates that when the heating time (30 minutes) and temperature (120° C.) are held constant the amount of creatine converted to creatinine is dependent upon the pH of the reaction mixture. The yields are 80% at pH 1, 98% at pH 2, and below 80% above pH 4.5. The type of buffer used to control the pH has but little effect on the yield between pH 1 and 3. The potassium chloride–hydrochloric acid and sodium citrate buffers are those described by Clark (*3*). The pH of the picric acid used was adjusted by means of sodium hydroxide. Contrary to the statement of Lambert (*10*), picric acid itself is not necessary for the conversion.

It is clear from these data that proper control of pH and temperature results in the saving of an appreciable amount of time. In addition, there is some evidence that lower yields of creatinine from creatine result under conditions requiring a prolonged heating time, although the authors' data do not justify a definite statement on this aspect of the problem.

As can be seen from Figure 4, the buffering capacity of picric acid drops off sharply at pH 2.4. This makes it necessary to use care in adjusting the pH of this solution and previously to adjust the pH of highly buffered samples. No adjustment of the pH is necessary for the dilute urine samples routinely measured.

Effect of Temperature. Figure 5 illustrates the effect of temperature and hydrogen ion concentration upon the reaction rate. At pH 2.0 all the reaction rates are faster than at pH 1.4, the pH of 1.17% picric acid in water. By adjusting the pH of the reaction mixture to 2.0 ± 0.05 it is possible to complete the reaction in 30

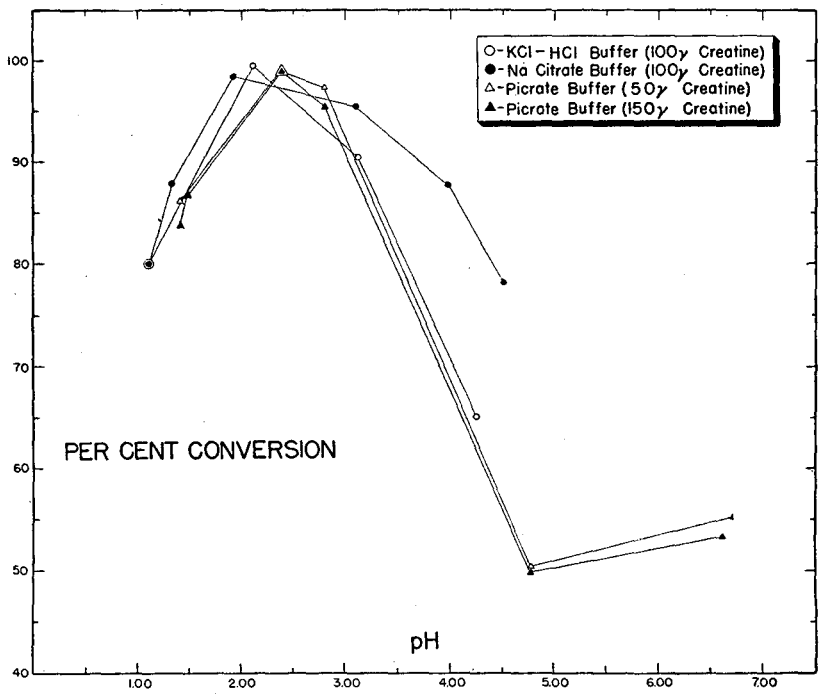

Figure 3. Effect of Hydrogen Ion Concentration on Rate of Conversion

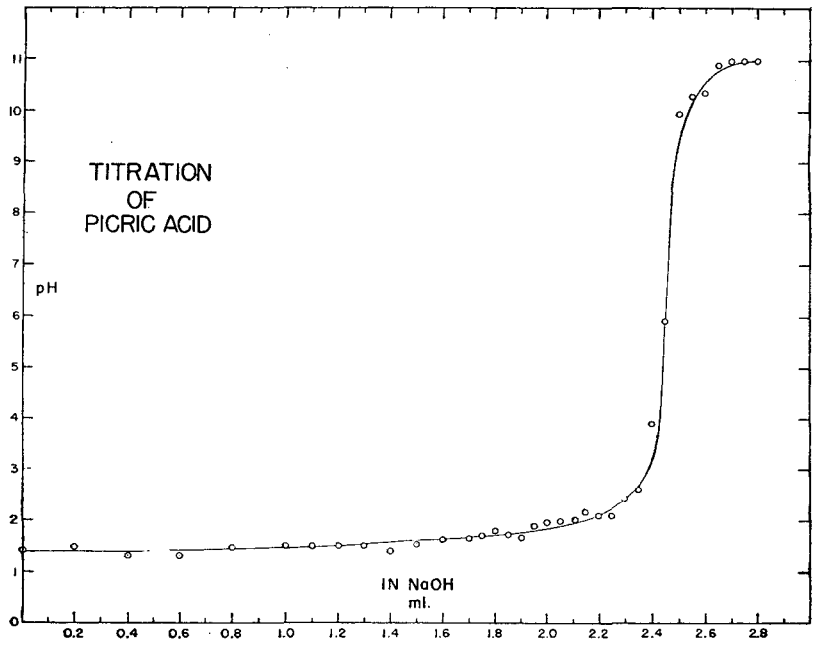

Figure 4. Titration of 50 Ml. of 1.175 % Picric Acid with 1 *N* Sodium Hydroxide

minutes at 120° C. Aside from the practical advantages of the shortened time, it would seem generally desirable to conduct a reaction under its optimum conditions.

Yield of Creatine from Creatinine. When pure standards are used, the conversion of creatine and creatinine has a mean value of 98.5% with a standard deviation of 1.09. These values were obtained from 42 routine checks distributed over a period of 2 years.

Recovery of Creatine Added to Urine. Table I lists the recovery of creatine added to urine, using the present method of analysis. These results illustrate that creatine can be measured quantitatively in the presence of urine by the procedure developed.

Identity of Reaction Product. In Figure 6 are shown the absorption spectra of the colored reaction products obtained from creatinine, creatine converted to creatinine, and a urine sample treated for the determination of creatine. The instrument was set to 100% transmittance using the reagent blank. The similarity of these curves is evidence that the final product measured in all cases is creatinine. Routine spectrophotometric measurements are made at 510 mμ rather than at the maximum (478 mμ) because the strong absorption of picric acid itself at the maximum would necessitate subtraction of an unnecessarily high reagent blank.

Creatinine and Creatine Content of Urine from Healthy Individuals. An inspection of the data obtained during the past year indicates a wide range of excretion of creatine and creatinine. For example, the creatinine excretion of 212 children between the ages of 6 and 18 years ranged from 0.281 to 2.05 grams in 24 hours. The creatinine excretion is probably best related to the muscle mass of the healthy child (*12*). These same children excreted between 0 and 0.428 gram of creatine per day. In the last 132 prenatal samples analyzed creatinine excretion in 24 hours ranged between 1.09 and 1.61 grams, while creatine excretion varied between 0.005 and 1.07 grams. Urine samples obtained within 3 months postpartum show a similar range in creatinine, but an excretion of creatine which is usually, though not always, markedly reduced. The healthy adult males tested excreted between 0 and 0.070 gram of creatine per day. A detailed analysis of these data is in progress and will be reported elsewhere.

DISCUSSION

The factors regulating the conversion of creatine to creatinine and those affecting the Jaffe color reaction (*9*) have been the subject of numerous studies during the past 40 years. The research has been directed toward an understanding of the reaction kinetics, a description of the precise nature of the color reaction, and the development of optimal analytical conditions. Space does not permit a complete review and detailing of the enormous literature, which has been far too often overlooked by investigators attempting to devise methods for the analysis of creatine and creatinine in biological fluids. The earlier literature has been reviewed by Hunter (*8*).

The reports of Edgar and co-workers (*4*, *5*) established the fundamental role of hydrogen ion concentration in establishing the final equilibrium between creatine and creatinine in aqueous solution and indicate, as well, that the hydrogen ion concentration controls the rate of conversion.

Table I. Recovery of Creatine Added to Urine

Creatine Added	Total Creatine Found[a]	Creatine Recovered	Recovery
γ	γ	γ	%
0	3.0	...	...
30	32.3	29.3	97.7
50	53.0	50.0	100.0
100	103.0	100.0	100.0
150	150.0	147.0	98.0
200	202.0	199.0	99.5
Mean	...	...	99.0

[a] This urine sample contained 0.792 mg. of creatinine per ml. It was diluted 1 to 20 before use.

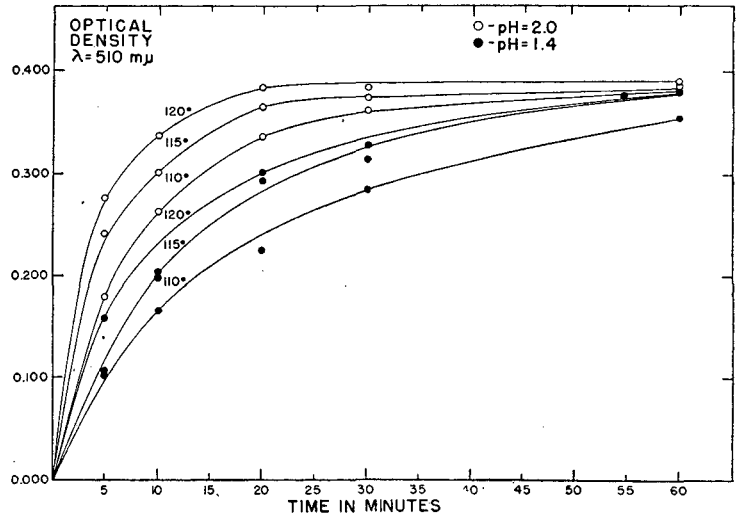

Figure 5. Effect of pH on Conversion of Creatine to Creatinine at Various Temperatures

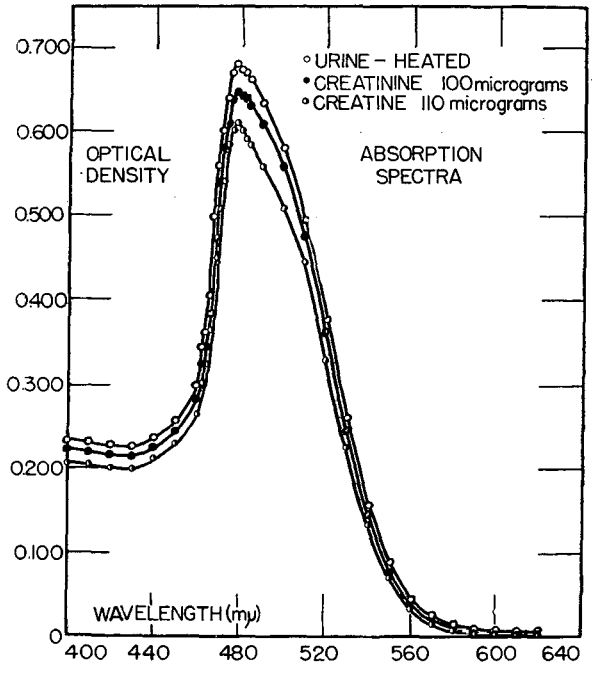

Figure 6. Spectral Absorption Curves

The authors' experiments were designed primarily to discover the optimum pH at which quantitative conversion of creatine to creatinine occurred. Independent of the type of buffer, this optimum point was found to lie near pH 2, a finding which could not have been predicted from the work of Edgar. In the light of this information, the Albanese (*1*) procedure has decreased, rather than increased, the reliability of the original Folin method, as has been pointed out by Lambert (*10*). However, contrary to the statement of Lambert, the effect is due, not to the presence of picric acid per se, but to the hydrogen ion concentration of picric acid solutions.

With the advent of modern photoelectric colorimeters and spectrophotometers, it is natural and appropriate that the Jaffe reaction be restudied in more precise terms. Curiously enough, part of the confusion introduced into the literature in recent years has stemmed from the use of filter-type colorimeters for this restudy. Regarding the determination of creatinine using picric acid, it has been repeatedly found (*10, 11*) that severe deviations from the Beer-Lambert equation occur and, furthermore, that two supposedly standardized instruments of the same manufacture may differ considerably from one another (*11*). Thus Lambert found that, in order to stay within the relatively short range of linearity using the Evelyn instrument, it was necessary to dilute urine samples, first to a constant specific gravity, and then again (about 1 to 20). By using the more precise Beckman instrument the range of linearity is so extended that a single dilution suffices for over 90% of all urines tested. Furthermore, it has been possible to study the conversion of creatine to creatinine, as well as the details of the color reaction itself, with much greater precision. This study, as reported above, has confirmed the original statements of Folin (*6, 7*) regarding the usefulness of the Jaffe reaction and has defined the analytical conditions in the more precise terms possible with modern instruments.

ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of Werner Jacobsen and Eleanor Clark during the early stages of this work. Determinations of carbon, hydrogen, and nitrogen were done by the Clark Microanalytical Laboratory, Urbana, Ill.

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Separation of Calcium from Magnesium by Oxalate Method

A Critical Study

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THE oxalate method for determination of calcium and its separation from magnesium dates from the earliest times of analytical chemistry (*7*). Fresenius (*4*) in 1868 stated that the precipitate of calcium oxalate is always contaminated by magnesium oxalate. It is therefore necessary to dissolve the precipitate in hydrochloric acid, and reprecipitate calcium oxalate with ammonia. Richards and collaborators (*16*) in 1901 showed that under certain conditions a single precipitation of calcium is sufficient. In the method described, a large amount of ammonium chloride is added. Calcium oxalate is precipitated by dropwise addition of ammonia to a calcium chloride solution containing oxalic acid and hydrochloric acid. After the precipitation an excess of ammonium oxalate is added.

Fischer (*3*) in 1926 published a paper, disparaging the work of Richards. He showed that magnesium oxalate is not precipitated from supersaturated solutions when special precautions are taken. In the determination of calcium, Fischer used but a slight excess of ammonium oxalate.

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Separation of calcium from magnesium by the oxalate method has been examined and double precipitation has been found necessary for accurate analysis. To avoid precipitation of magnesium from supersaturated solutions, a large excess of ammonium oxalate is used. In order to precipitate calcium quantitatively, the solution must be kept 4 hours at room temperature before filtering. The most favorable weighing form of calcium after the precipitation as oxalate is conversion to carbonate by ignition at 480° to 500° C. in air. A technical method is described, by single precipitation in acetic acid medium. To determine magnesium after the calcium determination, ammonium oxalate must be removed by evaporation of the solution to dryness and heating with a free flame. Bromine may be used as an oxidizing agent for ammonium oxalate in solution. Nitric acid does not remove oxalic acid.

Bobtelsky and Malkowa-Janowski (*2*) in 1927 described a procedure for the analysis of samples containing little calcium and comparatively large amounts of magnesium. They found that the solubility of magnesium oxalate was considerably increased by the addition of ammonium oxalate.

Herrmann (*8, 9*) in 1929 examined the precipitation of magnesium oxalate from supersaturated solutions. He found the crystallization to be extremely slow and to resemble the coagulation of a colloid. X-ray diagrams of crystalline calcium oxalate precipitated from solutions containing different amounts of magnesium showed that calcium oxalate and magnesium oxalate formed neither chemical compounds nor solid solutions.

In the course of time a large number of publications on calcium-magnesium determination have appeared (*1, 7, 12, 15, 21*). Further papers of less interest are not mentioned here. The large number of papers pertaining to the separation of calcium as the oxalate, and the equally large number of opinions expressed, are ample proof that the problem deserves further study. The present work has been carried out in order to elucidate some of the problems mentioned.

Table I. Weighing Form of Calcium

Expt. No.	Weighing Form of Calcium	Kept before Filtration, Hours	Calcium Found, Gram
1	$CaC_2O_4.H_2O$	1	0.1006
2		4	0.1006
3			0.1008
4		24	0.1010
5			0.1011
6	$CaCO_3$	1	0.0997
7			0.0998
8		2	0.0998
9			0.0998
10		4	0.0999
11			0.1000
12		12	0.1000
13			0.1000
14		24	0.1000
15			0.1000
16	CaO	1	0.1007
17		4	0.1008
18	Calcium oxalate	1	0.0996
19	titrated with	4	0.0998
20	0.1 *N* $KMnO_4$	24	0.0998

DETERMINATION OF CALCIUM IN CALCIUM CHLORIDE SOLUTIONS

The standard solution of calcium chloride was prepared by dissolving "Calcium *carbonicum praecipit.* for silicate analysis acc. to Smith," from Merck-Darmstadt, in pure hydrochloric acid, neutralizing with pure ammonia, and diluting with distilled water. Twenty-five milliliters of the standard solution contained 0.10000 gram of calcium.

The first aim of the investigation was to find out which weighing form of calcium is most favorable, after precipitation of calcium as the oxalate. The results are given in Table I.

Twenty-five milliliters of standard solution, diluted to 220 ml. with water, were heated to boiling, and 30 ml. of 0.1 molar ammonium oxalate solution were added dropwise with stirring. The precipitates settled rapidly and were easily filterable.

As seen from Table I, calcium oxalate monohydrate is not a good weighing form. This is in full agreement with the results of Sandell and Kolthoff (*17*). High results are often obtained if calcium is weighed as the oxide. Calcium oxide is hygroscopic and combines with carbon dioxide from the air. As mentioned by Hahn and Weiler (*6*), titration of calcium oxalate with potassium permanganate has a tendency to give low results.

The determination of calcium by ignition of calcium oxalate to carbonate in air was investigated by Willard and Boldyreff (*19*) who found that the oxalate is quantitatively converted into carbonate by ignition at 450° to 500° C. for 1 to 2 hours. Above 520° C. the carbonate is not stable. For the ignition an electric muffle furnace is required. When an accurate thermocouple is not available, the furnace can be adjusted to the right temperature by utilizing the melting points of silver chloride (455° C.) and lead chloride (501° C.).

On the basis of the results given in Table I, calcium was weighed as the carbonate in all later experiments. The determinations were carried out as follows:

After precipitation the solution was allowed to stand at room temperature for at least 4 hours, and then filtered through a porcelain filter crucible. The precipitate was washed with hot ammonium oxalate solution—3.0 grams of ammonium oxalate monohydrate per liter—until the washings gave no chloride reaction. The crucible was placed in the drying oven at 105° C. for at least 0.5 hour, and then in the electric furnace at 480° to 500° C. to constant weight (40 minutes + 20 minutes).

It is important that the precipitate be dry when placed in the electric furnace. Moist precipitates tend to be contaminated with a grayish cast of carbon after ignition, possibly due to catalytic effect of superfluous water vapor. Willard and Chan (*20*) state that particle size is a contributing factor in carbon contamination, this contamination being more evident with increasing particle size.

In precipitating calcium as oxalate, the following factors are important for the analytical results:

The concentration of the solution should be low; 0.01 molar solutions seem to be an appropriate dilution.

The interval between precipitation and filtration is of greatest importance. The precipitates have to stand 4 hours at room temperature before filtration. This is in full agreement with the statement of Richards (*16*).

The hydrogen ion concentration of the solution is an important factor. According to the literature (*5, 11*), calcium oxalate should be quantitatively precipitated from dilute acetic acid solutions. However, when the solution contains even relatively small amounts of a strong acid, such as hydrochloric acid, the precipitation is no longer quantitative. Even from a solution containing free oxalic acid, calcium oxalate cannot be quantitatively precipitated.

Some experiments have been carried out on the precipitation from dilute acetic acid. The results given in Table II prove that calcium oxalate can be quantitatively precipitated from dilute acetic acid, when the precipitant is added in excess, and liquid and precipitate are kept for at least 4 hours at room temperature before filtering.

In order to obtain precipitates with grain size convenient for filtering, the precipitation should be carried out in neutral or acetic acid medium. In dilute ammonia, a precipitate is obtained, adhering strongly to the beaker and to the stirring rod.

Table II. Precipitation from Dilute Acetic Acid

Expt. No.	After Precipitation: Molarity of Solution As to $(NH_4)_2C_2O_4$	As to CH_3COOH	pH of soln.	Kept before filtration	Calcium Found, Gram
21	0.002	0.002	4.6	4 hours	0.1000
22		0.002	4.6	Overnight	0.1000
23		0.004	4.2	4 hours	0.0999
24		0.004	4.2	Overnight	0.0998
25		0.010	3.8		0.0998
26		0.020	3.6		0.0999
27	0.01	0.004	4.6	Overnight	0.1000
28		0.01	4.3		0.1000
29		0.02	3.7		0.1000
30		0.03	3.6		0.1000
31		0.04	3.6		0.1000
32		0.05	3.5	4 hours	0.0999
33		0.05	3.5	Overnight	0.1001
34		0.10	3.4	4 hours	0.1000
35		0.10	3.4	Overnight	0.1000
36		0.20	3.3		0.1000

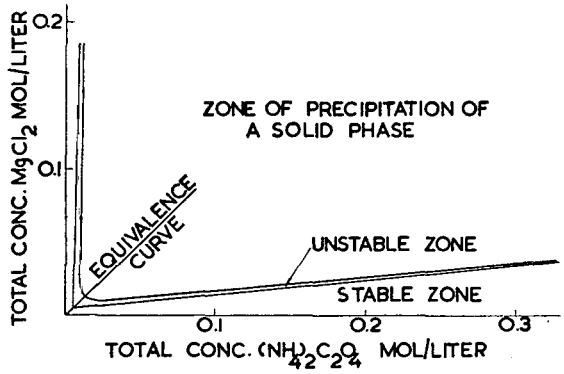

Figure 1. System Magnesium Chloride–Ammonium Oxalate–Water in Neutral Solution

The concentration of ammonium salts should not be unnecessarily large. With increasing ionic strength of the solution, the solubility of calcium oxalate will be increased in agreement with the Debye-Hückel theory. This increase in solubility will be shown by the diminution of the rate of crystallization (increasing "period of induction"). The addition of ammonium chloride is not only unnecessary but has an unfavorable effect on the analytical results. The effect of addition of ammonium chloride will be seen from Table III. To make sure that the increased solubility was not due to hydrolysis, caused by the ammonium chloride, ammonia was added in some of the experiments until a red color of phenolphthalein appeared.

The sensitivity of the oxalate method is good. Reliable results are obtained in the analysis of as little as 0.01 gram of calcium precipitated from a total volume of 250 ml. In the experiments, given in Table IV, the liquid was 0.01 molar as to ammonium oxalate after precipitation.

In experiments 1 to 70 the calcium oxalate was precipitated by the addition of 0.1 molar ammonium oxalate solution to a neutral solution of calcium chloride, or to dilute acetic acid containing calcium chloride. The precipitation can also be carried out by neutralizing an acid solution of calcium chloride and oxalic acid with dilute ammonia to the change of color of methyl red or phenolphthalein (*16*), or by adding the sample slowly to a neutral solution of ammonium oxalate (inverse precipitation). Results of experiments on these precipitation methods are given in Table V.

EXAMINATION OF MAGNESIUM OXALATE SOLUTIONS

The coprecipitation of magnesium oxalate with calcium oxalate is the result of adsorption and occlusion. Magnesium oxalate is slightly soluble in water, and the solutions are but partly dissociated. It was therefore of interest to examine the magnesium oxalate solutions under conditions similar to those existing after the precipitation of calcium oxalate in the analysis of calcium.

Solutions of magnesium chloride and ammonium oxalate were prepared. For each experiment a certain amount of magnesium chloride solution was diluted with water and heated to boiling. Ammonium oxalate solution was then added, and the mixture was left for 24 hours at room temperature. In some of the experiments a little magnesium oxalate was precipitated after that period. Where no solid phase had appeared, the solutions were heated to boiling, kept boiling for some minutes, and afterward left for 6 to 12 hours at room temperature. Some of these solutions then gave a precipitate; others gave no precipitate. In some cases the precipitation was carried out by adding the magnesium chloride solution to a hot solution of ammonium oxalate. Figure 1, giving the results, makes clear why inverse precipitation in some cases was more convenient.

The system magnesium chloride–ammonium oxalate–water is represented by Figure 1. By 64 precipitation experiments it was possible to draw the boundary lines between "zones":

Stable zone. No solid phase is precipitated.
Unstable zone. A solid phase is precipitated after boiling.
Zone of precipitation of a solid phase. A precipitate is formed within 24 hours after mixing.

In Figure 1 one will recognize Fischer's supersaturated solutions (*3*), equimolar solutions of magnesium chloride and ammonium oxalate. The solutions of relative supersaturation 4 are repre-

Table III. Effect of Ammonium Concentration

Expt. No.	Molarity of Soln., $(NH_4)_2C_2O_4$ after Precipitation	Total Concn. of NH_4Cl, Mole/Liter	pH after Precipitation	Kept before Filtration	Calcium Found, Gram
37	0.002	0.05	6.7	4 hours	0.0998
38		0.05	Phenolphthalein		0.0998
39		0.10	6.6		0.0998
40		0.10	Phenolphthalein		0.0998
41		0.20	6.3		0.0995
42		0.20	Phenolphthalein		0.0997
43		0.50	6.1		0.0988
44		0.50	Phenolphthalein		0.0991
45	0.01	0.05	6.7	4 hours	0.1000
46		0.10	6.6		0.0999
47		0.20	6.3		0.0999
48		0.50	6.1		0.0997
49	0.002	0.05	6.7	Overnight	0.1000
50		0.05	Phenolphthalein		0.1000
51		0.10	6.6		0.0999
52		0.10	Phenolphthalein		0.1000
53		0.20	6.3		0.0999
54		0.20	Phenolphthalein		0.1000
55		0.50	6.1		0.0996
56		0.50	Phenolphthalein		0.0996
57	0.01	0.05	6.7	Overnight	0.1001
58		0.10	6.6		0.1001
59		0.20	6.3		0.1000
60		0.50	6.1		0.1000

Table IV. Effect of Standing

Expt. No.	Kept before Filtration	Ca in Solution Mg.	Calcium Found, Mg.
61	4 hours	20.0	19.9
62		10.0	9.8
63		1.0	0.7
64		0.5	0.2
65		0.2	0
66	Overnight	20.0	20.0
67		10.0	9.9
68		1.0	0.8
69		0.5	0.3
70		0.2	0

Table V. Method of Precipitation

Expt. No.	Method	Kept before Filtration	Calcium Found, Gram
71	Neutralization with NH_3	4 hours	0.1000
72		Overnight	0.1000
73	Inverse precipitation	4 hours	0.0999
74		Overnight	0.1001

sented by the intersection between the "equivalence curve" and the boundary line of the unstable zone.

In Figure 1 one will further recognize the increased solubility of magnesium oxalate in excess of ammonium oxalate (*2*).

In the course of time several conclusions as to the nature of magnesium oxalate solutions have been made (*10, 14, 18*). No reliable physical investigation has been carried out, however, to make the problems clear.

On the basis of Figure 1 one may imagine the nature of these solutions to be as follows:

1. Solutions containing approximately equimolar amounts of magnesium chloride and ammonium oxalate. The solutions contain small amounts of magnesium ions and oxalate ions. By increasing the concentration, an increasing part of the magnesium oxalate will be present as undissociated magnesium oxalate. One may imagine that part of the undissociated magnesium oxalate will form larger aggregates, groups of molecules $(MgC_2O_4)_n$. These aggregates, being relatively stable, form supersaturated solutions. Certain dyestuffs may be absorbed to the surface of these aggregates, having a stabilizing action (*3*). The aggregates are broken up by boiling. If precipitation takes place after standing at room temperature, one may imagine that the crystalline structure designates the most stable stage of a succession of slow reactions. Therefore the precipitation of magnesium oxalate crystals will take place so slowly that the crystallization largely resembles the coagulation of a colloid (*3*).
2. Solutions containing a considerable excess of magnesium chloride. Part of the magnesium will be present as undissociated magnesium oxalate, and the solution contains but very little free oxalate ion. This phenomenon is also shown by the increased solubility of calcium oxalate in magnesium chloride solutions.
3. Solutions containing a considerable excess of ammonium oxalate. The solubility phenomena indicate the presence of a complex, $[(NH_4)_2C_2O_4]_m.[MgC_2O_4]_n$. Ammonium seems to be a component of the complex, the solvent action of ammonium oxalate being considerably greater than that of oxalic acid (*2*). The magnesium ammonium oxalate complex is relatively stable, may be submitted to boiling, and is by far more soluble than magnesium oxalate. From solutions containing an excess of ammonium oxalate, the precipitation of magnesium by phosphate or oxine is incomplete, and in some cases no precipitation at all can be obtained.

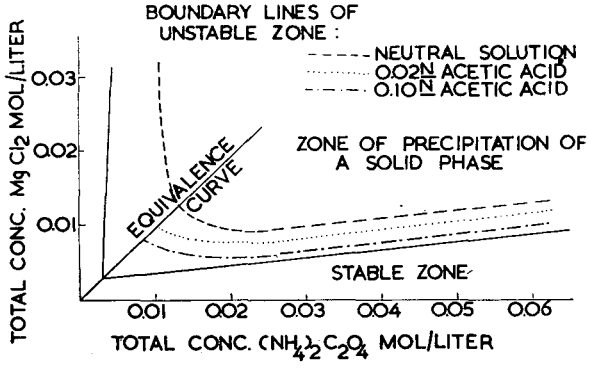

Figure 2. System Magnesium Chloride–Ammonium Oxalate–Water in Neutral and Acetic Acid Solutions

The precipitation experiments were repeated in acetic acid medium (Figure 2). Figure 2 shows that the boundary line of the stable zone is not altered by addition of acetic acid. The boundary line of the unstable zone, however, is shifted downward in the figure. This may be explained by supposing that the $(MgC_2O_4)_n$ aggregates are less stable in presence of hydrogen ions. The effect in many ways resembles that of the flocculation of a colloid by addition of an electrolyte.

SINGLE OR DOUBLE PRECIPITATION OF CALCIUM OXALATE FROM CALCIUM MAGNESIUM SOLUTIONS

A number of experiments have been made in order to find the best conditions for separation of calcium from solutions containing calcium and magnesium as the chlorides. Any contamination by magnesium of the calcium oxalate precipitates causes errors in the apparent analytical results on calcium. Therefore determination of the magnesium content of the ignited calcium carbonate was carried out in but a few experiments (Nos. 125 to 134). For those magnesium determinations the spectrochemical method was chosen.

Table VI. Fischer's Method

Expt. No.	Ca in Solution, Gram	Mg in Solution, Gram	Ca Precipitated, Gram	Ca Post-precipitated, Gram	Total Calcium, Gram
75	0.1000	0.0608	0.0965	0.0048	0.1013
76	0.1000	0.0608	0.0980	0.0028	0.1008
77	0.1000	0.1216	0.0949	0.0066	0.1015
78	0.1000	0.1216	0.0967	0.0042	0.1009

Table VII. Effect of Excess Ammonium Oxalate

Expt. No.	Mg Present, Gram	Total AmOx Added, Grams	Calcium Found, Gram
79	0	1.0	0.1001
80	0.0243	2.2	0.1003
81	0.0486	3.6	0.1004
82	0.0730	5.0	0.1005
83	0.0972	6.4	0.1006
84	0.1216	7.8	0.1012

Table VIII. Inverse Precipitation with Excess Ammonium Oxalate

Series No.	Expt. No.	Mg. in Solution, Gram	Total Ammonium Oxalate, Grams	Kept at Room Temp. before Filtration	Calcium Found, Gram
I	85	0.0243	1.25	4 hours	0.1004
	86	0.0365	2.0		0.1004
	87	0.0486	2.7		0.1004
	88	0.0608	3.4		0.1005
	89	0.0243	1.25	Overnight	0.1008
	90	0.0365	2.0		0.1005
	91	0.0486	2.7		0.1005
	92	0.0608	3.4		0.1002
II	93	0.0243	2.0	4 hours	0.1002
	94	0.0608	4.1		0.1005
	95	0.0243	2.0	Overnight	0.1002
	96	0.0608	4.1		0.1006
III	97	0.0243	2.0	4 hours	0.1004
	98	0.0608	4.1		0.1003
	99	0.0243	2.0	Overnight	0.1003
	100	0.0608	4.1		0.1004
IV	101	0.0243	2.0	4 hours	0.1000
	102	0.0608	4.1		0.1003
	103	0.0243	2.0	Overnight	0.1002
	104	0.0608	4.1		0.1004
V	105	0	1.0	4 hours	0.0999
	106	0.0243	2.0		0.1002
	107	0.0608	4.1		0.1001
	108	0	1.0	Overnight	....
	109	0.0243	2.0		0.1003
	110	0.0608	4.1		0.1002
VI	111	0.0061	1.0	4 hours	....
	112	0.0122	1.3		0.1002
	113	0.0243	2.0		0.1004
	114	0.0486	3.4		0.1004
	115	0.0608	4.1		0.1004
	116	0.0608	4.1		0.1006
	117	0.1216	7.6		0.1007
	118	0.1216	7.6		0.1004
	119	0.1824	11.2		0.1005
	120	0.1824	11.2		0.1006

1. **Richards' Method** (*16*). This method is one of compensation, a small amount of calcium oxalate being kept in solution, and a corresponding amount of magnesium oxalate being occluded. The experiments described in Table III show why Richards did not succeed in precipitating calcium oxalate quantitatively.
2. **Fischer's Method** (*3*). In case the ratio of magnesium to calcium is relatively large, one must expect Fischer's method to give unreliable results, because magnesium withdraws oxalate ions, yielding undissociated magnesium oxalate. Therefore calcium is not quantitatively separated after 4 hours. Experiments 75 to 78 (Table VI) were carried out according to the directions of Fischer. If the results of Table VI are compared with the experimental part of Fischer's paper, it is clear that Fischer's method is one of compensation, as in the case of Richards' method; Fischer's method is the less reliable of the two.
3. **Precipitation with a Large Excess of Ammonium Oxalate.**

Experiments 79–84, Table VII, show that coprecipitation increases with increasing concentration of magnesium in the solution. Experiment 84 shows that the coprecipitation is largely increased under working conditions involving passage of the "zone of precipitation of a solid phase" (Figure 1).

4. **Inverse Precipitation with Large Excess of Ammonium Oxalate.** In experiments 85 to 120 (Table VIII) the solutions to be analyzed were added to solutions of ammonium oxalate. The different series were carried out under somewhat varying conditions:

I. Hot ammonium oxalate solution. Time of precipitation 2 minutes.

II. Like I, but the amount of ammonium oxalate was somewhat larger.

III. Very slow precipitation, 15 to 20 minutes. The ammonium oxalate solutions were kept at 95° C. during the precipitation. The precipitates were coarse grained.

IV. Precipitation of small primary particles. The samples taken and the cold ammonium oxalate solutions were mixed rapidly. The liquid was then heated to boiling and placed on the steam bath for 1 hour.

V. Cold solutions mixed rapidly, placed on the steam bath for 1 hour, and then heated to boiling.

VI. Cold solutions mixed rapidly, placed on the steam bath for 15 minutes with stirring.

In none of the experiments was coprecipitation completely avoided. Experiments V and VI gave precipitates of very fine grain size when but little magnesium was present, and difficulties as to filtering arose. In spite of the large excess of ammonium oxalate present in solution, magnesium salts have so much influence on the solubility of calcium oxalate that it affects the recrystallization.

5. **Precipitation in Acetic Acid Medium.** The experiments were carried out as follows:

A certain amount of acetic acid was added to the ammonium oxalate solutions, and then the samples were rapidly added. All the solutions were cold. After mixing, the beakers were placed on the steam bath for 15 minutes, with stirring, and then were allowed to stand for 4 hours at room temperature. Total volume was 250 ml. The results are given in Table IX.

Table IX. Precipitation in Acetic Acid Medium

Expt. No.	Mol. of Soln. HAc after Precipitation	Ca Present, Gram	Mg Present, Gram	Total Ammonium Oxalate, Grams	Calcium Found, Gram	Spectrochem.[a] MgO in $CaCO_3$, %	Ca[b] Correction Value, Gram	Mg Coprecipitated, Gram
121	0.02	0.1000	0.0122	1.3	0.1002	..	..	..
122	0.02	0.1000	0.0243	2.0	0.1005	..	..	..
123	0.02	0.1000	0.0365	2.7	0.1003	..	..	..
124	0.02	0.1000	0.0608	4.1	0.1005	..	..	..
125	0.02	0.1000	0.1216	7.6	0.1002	0.3–0.4	0.0999	0.0005
126	0.02	0.1000	0.1824	11.2	0.1001	0.1–0.2	0.0999	0.0002
127	0.05	0.1000	0.0243	2.0	0.1001	0.1	0.1000	0.0001
128	0.05	0.1000	0.0365	2.7	0.1001	0.1–0.2	0.0999	0.0002
129	0.05	0.1000	0.0608	4.1	0.1001	0.1–0.2	0.0999	0.0002
130	0.05	0.1000	0.1216	7.6	0.1001	0.5	0.0996	0.0007
131	0.05	0.1000	0.1824	11.2	0.1000	0.3	0.0997	0.0004
132	0.05	0.0500	0.1824	11.2	0.0499	0.2–0.3	0.0498	0.0003
133	0.05	0.0200	0.1824	11.2	0.0199	0.3–0.4	0.0198	0.0001
134	0.05	0.0200	0.2432	15.0	0.0199	0.2	0.0198	0.0001

[a] Found by spectrochemical analysis.
[b] Corrected value (by $CaCO_3$ found—MgO).

Table X. Precipitation with Ammonia

Expt. No.	Mg Present, Gram	Total Ammonium Oxalate Added, Grams	Kept before Filtration, Hours	Calcium Found, Gram
135	0	3.0	1	0.0997
136	0.0243	3.0		0.1001
137	0.0608	3.0		0.1003
138	0.1216	3.0		0.1004
139	0	3.0	4	0.1000
140	0.0122	3.0		0.1002
141	0.0243	3.0		0.1003
142	0.0608	3.0		0.1005
143	0.1216	3.0		0.1006
144	0.1824	3.0		0.1025

Experiments 127 to 134 prove that the method is applicable for most purposes, giving results of sufficient accuracy. The method has been examined for molar ratios of calcium to magnesium from 2.5 to 0.05. The coprecipitation of magnesium is but slight in all cases. Calcium oxalate is practically completely precipitated.

6. **Precipitation with Ammonia.** The procedure given by Kolthoff and Sandell (*11*, p. 358) has been examined. The experiments were, however, as distinct from the instructions prescribing double precipitation, carried out by single precipitation.

The method of precipitation is largely similar to that of Richards; however, ammonium chloride is not added. Nevertheless, the concentration of ammonium chloride after precipitation is considerable.

During the precipitation it became apparent that the more magnesium present in solution, the more ammonia had to be added before crystallization. This phenomenon was first observed by Blasdale (*1*). The reason is the somewhat increased solubility of calcium oxalate, less supersaturation, and longer period of induction. The results are given in Table X.

Table XI. Double Precipitation

Expt. No.	Ca Present, Gram	Mg Present, Gram	Calcium Found, Gram
145	0.1000	0.0122	0.0999
146	0.1000	0.0122	0.0998
147	0.1000	0.0486	0.0999
148	0.1000	0.0486	0.0998
149	0.1000	0.0973	0.0998
150	0.1000	0.0973	0.0998
151	0.0100	0.0973	0.0098
152	0.0100	0.0973	0.0097

Kolthoff and Sandell urge that the precipitate of calcium oxalate should be kept 1 hour only, before filtering. The experiments show, however, that calcium oxalate cannot be quantitatively precipitated after so short a period. From experiment 144 one may see that by operating with a fixed amount of ammonium oxalate (3.0 grams) some magnesium oxalate may be precipitated if the concentration of magnesium exceeds a certain limit.

7. **Double Precipitation.** Some experiments involving double precipitation were carried out.

The first precipitations were carried out as described in Section 6, the precipitates being kept 4 hours before filtration. For filtration a filter paper was used, and after washing, the calcium oxalate was dissolved in dilute hydrochloric acid. At the second precipitations 1.0 gram of ammonium oxalate was used, and the precipitates were left overnight before filtration.

From Table XI one will see that the results are somewhat low, but the method is reliable, giving results within ±0.2 mg. from the theoretical values.

REMOVAL OF AMMONIUM OXALATE

The presence of ammonium oxalate will prevent the precipitation of magnesium by phosphate or oxine. If a large excess of ammonium oxalate has been employed in the determination of calcium, the ammonium oxalate must be destroyed before determination of magnesium.

Most available textbooks maintain that oxalate can be destroyed by the aid of concentrated nitric acid. A number of experiments have proved, however, that nitric acid is not a sufficiently strong oxidizing agent for the destruction of oxalate. Neither is aqua regia. Ammonium oxalate may be completely destroyed by volatilization over a free flame, after evaporation of the solution to dryness.

Bromine may be used for the oxidation of ammonium oxalate in aqueous solution.

For each 5 grams of ammonium oxalate monohydrate present, the solution is evaporated to a volume of 100 to 150 ml. and transferred to an Erlenmeyer flask. After cooling to room temperature, 1 ml. of bromine is added, and a glass-tube condenser (30 to 40 cm.) is mounted on the Erlenmeyer flask. After vigorous shaking for some minutes, the Erlenmeyer is placed on the steam bath and heated with shaking until the color of bromine has disappeared. This procedure is repeated twice. (For 5 grams of ammonium oxalate 1 + 1 + 1 ml. of bromine must be added, and correspondingly 15 grams of ammonium oxalate require 3 + 3 + 3 ml. of bromine.) At last the liquid is heated without condenser to boiling for removal of superfluous bromine. It is of the greatest importance that the liquid be cold when bromine is added; the boiling point of bromine is +59° C.

By this procedure more than 95% of the ammonium oxalate may be removed without difficulty, and the method is probably the most time-saving of those available.

It has been known for a very long time that oxalic acid in aqueous solution can be destroyed by electrolytic oxidation (*13*). Experiments have confirmed that by operating at 70° C. with a direct current and platinum electrodes, the voltage being 12 to 14 volts, more than 95% of the oxalic acid of an aqueous solution was destroyed. At the same time the volume of the solution decreased to one fifth. However, a procedure applicable for analytical purposes has yet to be worked out.

RECOMMENDED PROCEDURES

By the precipitation of calcium oxalate from solutions containing calcium chloride and magnesium chloride, some magnesium oxalate will always be occluded. In accurate analysis, the precipitate should therefore be dissolved in hydrochloric acid and reprecipitated with ammonia. A large excess of ammonium oxalate prevents the precipitation of magnesium from supersaturated magnesium oxalate solutions.

By single precipitation satisfactory separation of calcium from magnesium can be obtained by the following methods:

If the sample does not contain more than 25% magnesium, the precipitation can be carried out in the usual way, by the neutralization with ammonia of a hydrochloric solution containing calcium ion and an excess of oxalate ion. Ammonium chloride should not be added.

If the sample contains from 25 to 90% magnesium, the precipitation can be carried out as follows: An amount of ammonium oxalate, the molar ratio as to magnesium present being 10 (but not less than 3.0 grams), is dissolved in 200 ml. of water, and the solution is cooled to room temperature. Then enough acetic acid is added to make the solution 0.05-molar. The solution to be analyzed, containing 100 to 150 mg. of calcium plus magnesium, is added from a pipet. The beaker is then placed on the steam bath for 15 minutes, with stirring, and is afterward kept 4 hours at room temperature.

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Preparation of Lycopene from Tomato Paste for Use as a Spectrophotometric Standard

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THE increasing commercial production of tomato products, particularly tomato sauce, puree, and paste, is leading to an increased interest in the control and improvement in the quality of these food materials. Suitable objective methods for measuring the chemical constituents in food products are constantly being investigated, and because the color of tomato products is often taken as a good criterion of their quality, an accurate method for measuring the pigments is of particular importance.

The pigments normally found in tomato products are lycopene and other carotenes, of which lycopene is predominant. The determination of lycopene requires the use of a spectrophotometer or colorimeter, and a sample of pure pigment is necessary in the preparation of calibration curves.

This paper describes a convenient, rapid method for the preparation of pure lycopene from tomato paste for use as a spectrophotometric standard, because lycopene is not available commercially at present. Strain (*7*) described the preparation of lycopene by combination of a partition in immiscible solvents and chromatographic methods. A reduction in the volume of the solvent was required before crystallization. Zechmeister *et al.* (*8*, *12*) used a method similar to that of Strain, while Zscheile and Porter (*14*) did not describe their method of preparation.

Tomato paste is a convenient source of material for the isolation of lycopene because it is finely divided, is readily available the year round, and has a high lycopene content. Although some varieties of tomatoes have been reported to contain as much as 300 to 400 micrograms of lycopene per gram, 23 strains varied from 32 to 111 micrograms per gram (*5*). The average value for lycopene found in 15 samples of tomato paste prepared in this laboratory was 410 micrograms per gram, ranging from 274 to 545 (all figures on a wet-weight basis).

Tomato paste appears to be a better source of material than

A new rapid method for the preparation of lycopene for use as a spectrophotometric standard is described. Neither partition in immiscible solvents nor chromatographic columns are used, but instead a simple extraction and crystallization in acetone and one recrystallization in methyl alcohol. No unusual precautions against deterioration were taken, except to work rapidly and avoid strong light. Quantitative absorption curves obtained by the use of lycopene prepared by this method approximate those of Zechmeister and Zscheile.

fresh tomatoes for isolation of lycopene; however, it has been subjected to high temperature, oxidation, and similar deteriorating factors and these conditions are known to cause a breakdown and isomerization of carotene pigments (*6*). Preliminary work on the determination of pigments in tomato paste, processed under pilot-plant conditions, indicated, however, that approximately 85% of the lycopene in the fresh tomato puree, as it came from the finisher, was retained in the canned paste after processing. Inasmuch as it is known that carotene undergoes isomerization as a result of processing, investigations were included to study these isomers in tomato paste and their effects on the isolation of pure lycopene.

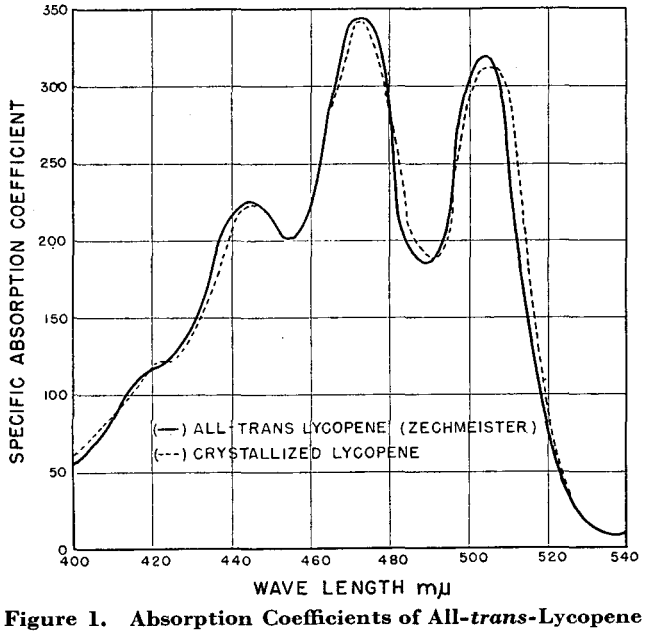

Figure 1. Absorption Coefficients of All-*trans*-Lycopene

The isomers of carotenes present in tomatoes are of particular interest, because they increase the difficulty of preparing spectrophotometrically pure lycopene. Zscheile and Porter (*14*), in a paper reporting the results of a study of the pigments of many strains of tomatoes, brought together for comparison the quantitative absorption curves of the ten most abundant carotenes, including the cis-trans isomers found in the fruit of tomatoes. These curves indicate how easily the very high peaks of the all-trans form of lycopene can be lowered by contamination with the cis forms of lycopene or other carotenes. Zechmeister and Pinckard (*10*) have recently reported six new isomers of lycopene in Pyracantha fruit, but apparently these were not found in tomato products. Phytofluene, a recently discovered colorless polyene found in tomato paste (*13*), has its main absorption at a lower wave length than lycopene and would not be likely to affect the shape of absorption curves of lycopene above 400 millimicrons even if it occurred in large quantities. In order to avoid the effect of isomers in the work reported here, recrystallization of the lycopene was continued until no further increase in the absorption coefficients occurred.

METHOD OF PREPARATION

To the contents of a 6- or 8-ounce can of tomato paste, having a bright red color, are added 250 ml. of a solution of 30% potassium hydroxide in methyl alcohol (*4*). The mixture is shaken at intervals until well mixed and placed at 5° C. overnight. Either the whole or a portion of the saponified paste is mixed with distilled water, and sufficient filter aid is added to disperse the paste conveniently, which is then spread on a thinly filter-aid-precoated filter paper (24 cm. No. 595 S&S) in a large suction funnel. The cake is washed with distilled water until it is approximately free of alkali, as shown by the almost colorless filtrate. Care should be taken to keep the cake covered with liquid. When the quantity of filter aid is properly adjusted, the flow of filtrate is copious, and washing may be completed in 10 to 15 minutes. If the cake separates from the funnel, the crack formed can be filled and sealed by a thin stream of a thick suspension of filter aid poured at the edge of the funnel. A tight seal is necessary for successful washing and extracting.

The lycopene is extracted from the cake on the filter, which is held tightly at maximum suction, by acetone in charges of 50 to 75 ml. Approximately 300 ml. of acetone per ounce of paste are required. To counteract the effect of decreasing temperature resulting from rapid evaporation of acetone under reduced pressure, the acetone is heated to 35° C. before extraction. The washing and extracting can be completed in 25 to 30 minutes and the first one or two charges may be discarded because they contain little lycopene. The glittering red crystals of lycopene start forming at once in the filtrate and can be filtered off immediately after extraction. However, a better yield with less effort is obtained by letting the extract stand at low temperature (as low as 0° F.) until the crystals settle to the bottom of a tall cylinder or bottle. Most of the solution containing carotenes other than lycopene can be decanted. Lycopene in acetone deteriorates slowly at low temperature.

An alternative method of extraction and saponification can be used. The paste is stirred in a blender with 1% metaphosphoric acid for 5 minutes, mixed with filter aid, filtered, and washed on a Hirsch suction funnel with distilled water. The use of metaphosphoric acid greatly hastens the filtration procedure. Acetone is poured on the cake in small charges, and 100 ml. of acetone are sufficient to extract the pigments completely from 5 grams of paste. After saponification, a reduction in the volume of the solvent, hexane or petroleum ether, is necessary to start crystallization. In either method the cake of filter aid seems to act somewhat as a chromatographic column but gives a much more rapid extraction than the column.

When crystals of lycopene are needed, they are rapidly filtered off on hard filter paper, washed with a little acetone, and dissolved in recently distilled chloroform (*2*). After being filtered again, they are recrystallized by addition of methyl alcohol (*3*). The crystals separate easily in a thick film on the hard paper and are then dried at low pressure (1 to 3 mm.) in a desiccator over calcium chloride at room temperature for 1 hour (*1*). No precautions against deterioration, such as the use of inert gas, were used, except to work rapidly and avoid strong light. Twenty milligrams of lycopene are dissolved in 3 ml. of chloroform, and hexane is added to a volume of 200 ml. This stock solution is used in preparing a solution in hexane for calibration or for determination of quantitative absorption curves by the Beckman spectrophotometer. One milligram per liter of the lycopene prepared by dilution of the stock solution was used for the determination of the absorption coefficients (Zscheile's equation for Beer's law was used in making the calculations).

The data were plotted on thin paper and superimposed on curves for lycopene found by other investigators. Figure 1 shows the curve for the absorption coefficients of all-*trans*-lycopene, crystallized once each from acetone and methyl alcohol and superimposed on a similar curve prepared by Zechmeister *et al.* (*9, 11*). Similar results were obtained with Zscheile's curve (*14*). The average values of the absorption coefficients from

three lots of tomato paste were 223 (range 223 to 225) at 445 to 446 millimicrons, 341 (range 338 to 345) at 473 to 474 millimicrons, and 310 (range 306 to 315) at 505 millmicrons, using slit widths below 0.05 mm. Further crystallization gave no higher values. On a column of either magnesia-filter aid (50-50 mixture, by weight) or lime (325-mesh), the purified lycopene appeared homogeneous.

The spectrophotometer was checked for wave-length calibration by use of lines of a mercury arc. Before its use as a standard for colorimetric work, the lycopene should be checked spectrophotometrically by the user.

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Measuring Color and Turbidity of White Sugar Solutions

Development of Photoelectric Method and Apparatus

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In 1935 a photoelectric instrument was developed for measuring the color of solutions of raw sugar and other dark-colored liquors and sirups. Continued investigative work led to the development of another photoelectric device for measuring the color of solutions of white sugar and light-colored sugar liquors. This instrument has eliminated practically all the difficulties associated with the visual method of color determination formerly in use for white sugar solutions. In determining the color of white sugar solutions, a single transmittance measurement in the visible spectrum is representative of both color and turbidity. In white sugar solutions, even small amounts of turbidity often have a pronounced effect on the transmittance reading. Because this turbid material cannot be readily removed by usual laboratory filtration methods, it is necessary to correct for it. In the instrument that was developed, transmittance measurements are made at two different wave lengths. One reading, taken at the blue end of the visible spectrum, is representative of the total light absorption by both the color and turbidity in the solution. A second reading, taken in the near infrared portion of the spectrum, is not influenced by the color of the sugar solution and therefore gives a direct indication of turbidity. Thus by subtraction of the infrared reading from the blue reading on a $-\log T$ basis, a direct indication of the solution color is obtained. This determination of color is accurate, reproducible, and rapid and can be made by nontechnical personnel. The development of this instrument, and instruments previously described by the authors, has placed all color determinations in this laboratory on a photoelectric basis.

THE determination of color is an important analytical procedure in a cane sugar refinery, not only from the standpoint of routine process control, but also in the evaluation of the quality of the refined sugar. This significance of color has been recognized in this laboratory for a great many years and the subject has been investigated in considerable detail with the view of ultimately replacing all inaccurate visual procedures with photoelectric methods and thereby eliminating subjective errors from such measurements. A previous paper (*5*) described the development of a photoelectric colorimeter for measuring the color of solutions of raw sugar and of refinery liquors and sirups. A second publication (*3*) described a photoelectric reflectometer for determining the relative color of brown sugars.

Following these developments, attention was directed to photoelectric measurement of colors of practically water-white solutions of granulated sugars. A device employing long cylinders (*5*) was suitable only for determining the total light absorptions of the solutions and could not be used for measuring color alone because of the interfering effect of small amounts of turbidity. Therefore the problem was approached from a different direction in an attempt to develop an instrument that would readily measure turbidity as well as total light absorption, so that suitable correction could be made for turbidity.

PROBLEMS INVOLVED IN MEASURING COLORS OF WHITE SUGAR SOLUTIONS

Prior to the development of the compensating photoelectric colorimeter described herein, colors of white sugar solutions were determined visually in this laboratory. This method involves comparing, in two long clyinders, a 50% solution of the white

sugar with a mineral solution standard having an arbitrarily assigned color value. The method (*11*) gives colors that are directly proportional to $-\log T$, even though arbitrary C and H color units are employed.

Because visual colors obtained by this method are subject to variables that introduce numerous errors, the method is considered inaccurate and inadequate for control and investigative purposes. The principal error is probably due to the turbidity occasionally found in solutions, which makes it very difficult to obtain a close match in color.

As pointed out by Zerban and Sattler (*12*) and borne out by the authors' experience, turbidity cannot be removed from white sugar solutions by filtration through kieselguhr and similar media because some of the color is also removed. This results in appreciable errors. The problem of filtration without color removal was investigated by Peters and Phelps (*10*), who described a method of using asbestos as a filter medium. The preparation of the asbestos pads and the slow filtration of the high density white sugar solutions were found too time-consuming for use in routine color determinations. Considerable difficulty occasionally developed in completely eliminating the fibers and slight haze associated with asbestos filtration. Filtration therefore appeared to be out of the question as a means of improving the visual method or of eliminating the effect of turbidity on photoelectric measurements of color.

PRINCIPLES OF COMPENSATING PHOTOELECTRIC COLORIMETER

The present investigation of photoelectric means of measuring the solution color of white sugars was directed toward the development of some method of correcting for the small amount of turbidity in the solution, so that filtration could be avoided. It was determined that photoelectric measurements of the light absorption of a white sugar solution at two different wave lengths offered a means for correcting or compensating for the turbidity present.

The method developed is based primarily on photoelectric measurement of the light absorption of a sample at two different wave lengths and the proper correlation of these two results. A color measurement at the shorter wave lengths in the blue region of the visible spectrum gives a measure of the total light absorption—color plus turbidity; a measurement at the longer wave lengths in the yellow or red part of the spectrum is primarily indicative of turbidity. By proper correlation of these two measurements, it is possible to apply a turbidity correction to the total absorption measurement and obtain a value indicative of the color only.

In the preliminary investigative work the first color filters employed were Corning No. 503 Dark Theatre blue and Corning No. 351 yellow shade yellow glass filters. However, although the blue filter gave a reading indicative of total light absorption, readings through the yellow filter were influenced to some extent by the color of the sample. Consequently, a series of curves was developed, based on readings with both filters, to provide a turbidity correction that could be applied to the reading with the blue filters to give color alone. An instrument, using these two filters, was employed in the laboratory for several months and gave satisfactory performance, but a simpler approach to the problem of turbidity correction was desired, by which the correction curves could be eliminated, the time required to arrive at a color result reduced, and a more direct reading of color obtained.

The investigation was therefore continued in order to locate another filter by which a direct measure of turbidity, unaffected by color, could be obtained. It was finally found that Eastman filter Wratten No. 88A infrared would provide such a measurement, and it was selected for use in the instrument. This is a gelatin filter which is mounted between glass plates and used in the same manner as the Corning filter. The blue filter was also changed because it was reportedly somewhat unstable and Corning No. 554 (now No. 5543) H.R. Lantern blue glass filter was substituted. The combination of these two replacement filters avoided the previous complex relationship and provided the desired simple subtractive means of correcting for turbidity.

The spectral characteristics of the selected filters are described in the bulletins of the manufacturers. The Corning No. 554 blue filter is a rather broad band filter in the blue region of the spectrum and has maximum transmittance at about 420 millimicrons. The Wratten No. 88A infrared filter does not transmit below about 720 millimicrons but has high transmittance in the near infrared.

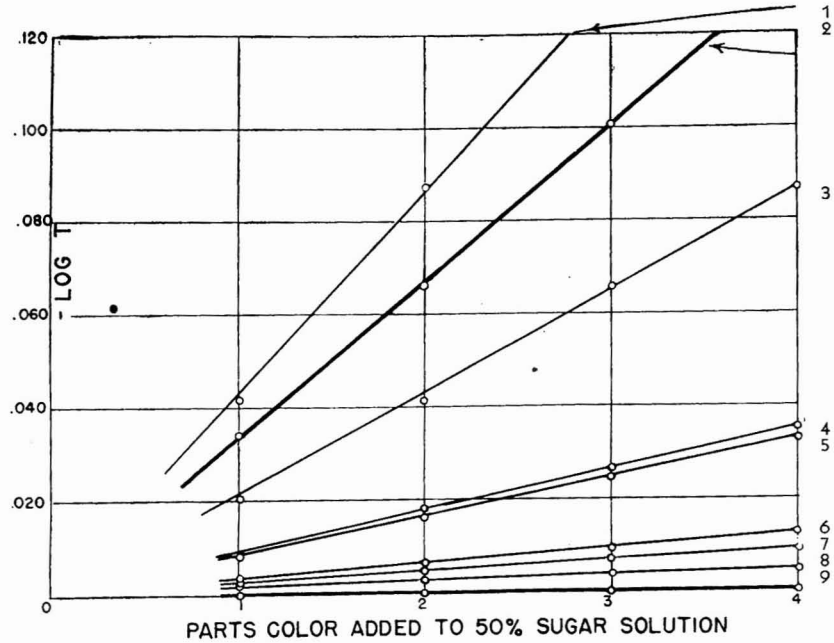

Figure 1. Photoelectric Transmittance Measurements with Different Color Filters on Sugar Solutions of Varying Color

1. No. 511 violet
2. No. 554 Lantern blue
3. No. 503 D Theatre blue
4. No. 428 light blue green
5. No. 401 Sextant green
6. No. 351 yellow shade yellow
7. No. 349 Lantern yellow
8. No. 243 Signal red
9. No. 88A infrared

The selection of the No. 88A infrared filter was based on results obtained with two series of specially prepared test samples. One series included turbidity-free samples of increasing color, while the other series included color-free samples of increasing turbidity. For the first series, a stock color solution was prepared by carefully clarifying a typical refinery product such as washed raw sugar liquor and adjusting to a 50% concentration. This stock solution was then added incrementally to portions of a specially decolorized and clarified 50% solution of confectioners' sugar. The instrument readings obtained with these solutions, using different color filters, are plotted in Figure 1.

Of all the filters tested, the Wratten 88A infrared filter was the only one that gave no instrument response to color. Its use would therefore permit the desired simple subtractive turbidity correction, provided an adequate response could be obtained to turbidity.

For the second series a stock turbid solution was made by adding bentonite to a specially decolorized confectioner sugars' solution; the suspension was filtered through filter paper to remove the larger particles that might have caused difficulty by settling. This stock solution was added in increments to other portions of the clear, colorless confectioners' sugar solution used with the

first series. The results with this series of tests are plotted in Figure 2.

It is evident from Figure 2 that the instrument responded to turbidity regardless of the type of color filter employed. In this case, as was true in Figure 1, larger readings were obtained in the blue and violet portions of the spectrum than in the red and yellow regions. Nevertheless, a satisfactory turbidity response was obtained using the Wratten No. 88A infrared filter.

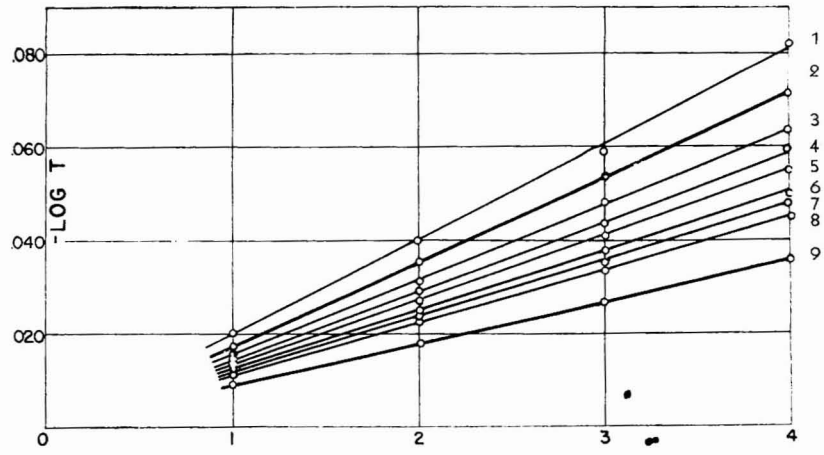

Figure 2. Photoelectric Transmittance Measurements with Different Color Filters on Sugar Solutions of Varying Turbidity

1. No. 511 violet
2. No. 554 Lantern blue
3. No. 503 D Theatre blue
4. No. 401 Sextant green
5. No. 428 light blue green
6. No. 351 yellow shade yellow
7. No. 349 Lantern yellow
8. No. 243 Signal red
9. No. 88A infrared

In developing these turbidity relationships, a number of turbid media were considered. Bentonite was finally selected as the most suitable and was used in these as well as subsequent test samples. The low concentrations employed caused no problem with Tyndall beam effect and gave very stable solutions for short periods of time. Furthermore, at these low concentrations, bentonite was considered more or less comparable to the turbid material found at times in white sugar solutions. In similar experimental work, bentonite has been used widely by other investigators (*1*, *7*, *13*).

Turbidity measurements of this nature are generally relative. In this case such measurements were satisfactory because ratios between readings with different filters were being determined. This approach has apparently been justified by the successful application of the relationships developed and by the satisfactory results obtained in practice.

Other investigators have also utilized the principle of measurements at two different wave lengths to obtain the color of white sugar solutions. Keane and Brice (*6*) developed a method and an instrument in which readings were made in the blue and red portions of the visible spectrum. The two filters employed were Corning No. 428 light blue-green color filter and Corning No. 245 Signal red color filter. As pointed out by Nees (*9*), the Keane and Brice method makes two assumptions: that the absorption of the light passing the red filter is not affected by the color of the solution but only by the turbidity present in the solution, and that turbidity has the same effect on the light passing through the blue filter as it does in the case of the red filter. From Figures 1 and 2, it appears that neither of these assumptions is completely valid. Nees (*9*) improved upon this method, using a Lange colorimeter with the blue and yellow filter provided with the instrument. He developed a mathematical relationship based on the fact that the light transmitted through both filters would be affected by both color and turbidity, and thereby was able to express the absorption in terms of color alone. Nees' use of the yellow filter did not overcome the objection that the yellow light is affected by both color and turbidity.

Morse and McGinnis (*8*) developed a method using the Lumetron photoelectric colorimeter. Two readings were made on this instrument, using the same filters employed by Keane and Brice, and a mathematical relationship was obtained between the two readings. In this way, they were able to determine a color index and a turbidity index for sugar solutions. Although this procedure was an improvement over previous methods, it still did not provide the simple subtractive relationship obtained in the present investigation.

DESCRIPTION OF INSTRUMENT

Following the principles outlined in the foregoing paragraphs and using the two filters indicated, an experimental instrument was constructed and used on a trial basis for several months. Very satisfactory results were obtained and another more refined colorimeter was therefore designed and constructed, which has given satisfactory service during 10 years' constant use in this laboratory. The general appearance of this instrument (*4*) is shown in Figure 3.

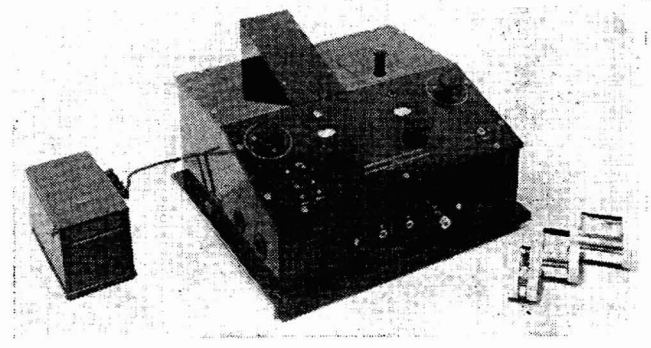

Figure 3. Photoelectric Colorimeter

Optical Arrangement. The optical arrangement is shown in Figure 4. The light source is a standard 110-volt alternating current, 200-watt projection lamp housed in an insulated, ventilated compartment. The lamp is located in the center of the instrument and projects two equal beams of light in opposite directions. Use of a single light source in this manner avoids errors due to voltage fluctuations and other related variables, as both photocells are affected identically.

One beam passes through the glass window of the lamp housing, the proper optical filter, and the absorption vessel containing the sample, and finally impinges on the measuring photocell. In the other direction, the light path to the comparison photocell is similar, except that no absorption vessel is used.

The filters are mounted in a metal frame which can be moved by means of an exterior lever, so that either the blue or infrared filters can be positioned in the two light paths as desired. All apertures are fixed in size and position, except the aperture in front of the comparison photocell. This aperture is adjustable in size, so that use of an absorption vessel in the light path to this photocell can be avoided.

Photoelectric Circuit. The photoelectric circuit is based on that used in the Holven-Gillett photoelectric colorimeter (*5*). Essentially, it utilizes two carefully matched Weston photogenerative type cells that are shunted by fixed resistances and connected in opposition in a potentiometer type circuit. One of the shunts is a calibrated slide-wire which permits obtaining percentage light absorption directly, so that colors can be expressed in $-\log T$ units. A galvanometer is used to indicate condition of balance between the two cells.

In utilizing the basic principles of this circuit, certain features were added and modifications made, so that it was more suitable

for measuring the extremely small amounts of color in white sugar solutions, and so that operation of the instrument was simplified. The circuit modifications are illustrated in Figure 5.

The first circuit indicates the principal modification that was made to increase the sensitivity of the instrument. An additional fixed resistance approximately equivalent to the resistance of the slide-wire was inserted in each shunting circuit. This had the effect of spreading only half of the absorption range over the entire slide-wire scale and thus essentially doubling the sensitivity. The range of the the slide-wire was reduced from 0 to 100% light absorption to 0 to 50% absorption, so that a reading of, say, 5 on the uniformly calibrated 0–100 slide-wire actually represented 2.5% absorption.

Another circuit modification, illustrated in the center diagram of Figure 4, shows the insertion of a second slide-wire in the circuit. By this change, one slide-wire could be used with the blue color filters and another with the infrared filters. This was done primarily for two reasons. In the first place, the photoelectric response for a given turbidity is different at one wave length than at another and therefore a different $-\log T$ scale would be required with different color filters if the readings are to be placed on a comparable basis. With two slide-wires, one could therefore be calibrated for the blue filters and the other for the infrared filters, thus avoiding two scales on a single slide-wire. In the second place, not only was it simpler to obtain absorption readings, but one reading could be retained while the second one was being taken. In actual operation, the slide-wire involved is automatically placed in the circuit in accordance with the positioning of the filters associated therewith. In other words, when the blue filters are in the light path the slide-wire calibrated for such readings is in circuit; when the infrared filters are used, the slide-wire calibrated for use with the latter filters is in circuit. This is accomplished automatically by means of contact switches on the movable frame holding the filters.

Two adjustable rheostats have been included in the shunt circuit to the measuring photocell. One is used with one slide-wire to balance the circuit when the blue filters are in position. The second is used with the other slide-wire when the infrared filters are in position. This circuit modification greatly simplifies the balancing procedure.

A further circuit modification relates to the elimination of the use of a balancing standard during the initial balancing of the instrument. In most colorimeters, it is necessary to balance the instrument initially against some transmittance standard such as distilled water, before readings are made on the sample. This is generally accomplished by first filling the absorption vessel with the distilled water, balancing the device, replacing the distilled water standard with the sample, rebalancing the instrument, and then taking the reading. This procedure is time-consuming and a nuisance, particularly with high density samples such as 50% white sugar solutions, because of the excessive rinsing of the vessel that is required and the difficulty of avoiding striations in the sample, which affect the transmission of light.

The necessity for following such an involved method of initial balancing was avoided by the addition of compensating fixed resistances in the circuit. These resistances provided a condition of initial balance, without any vessel, identical to the condition of balance that would have resulted if the absorption vessel containing the balancing standard had been in the light path to the measuring photocells. The balancing standard for establishing these resistance values was a specially prepared clear, colorless sugar solution, which was used instead of distilled water as it more closely approached the transmissivity of the sample solutions. The arrangement of these compensating resistances in the circuit is illustrated by the third diagram in Figure 5. In balancing the instrument initially, these resistances are placed in circuit simply by means of a cam key switch. One of these compensating resistances is used with the blue filters and another with the infrared filters.

The values of these resistances obviously depend on the length of column of standard or sugar solution employed. A separate pair of resistances is employed for each of the three vessels (3, 6, and 9 cm.) employed with this instrument. The proper balancing resistances are selected by means of a jack plug connection at the front of the instrument.

The reference standard which is usually placed in the light path to the comparison photocell was also eliminated by using an adjustable aperture to restrict the light impinging on this second cell to the same extent as would exist if an absorption vessel containing the reference standard had been in the light path.

Absorption Vessels. The absorption vessels were made from 5-cm. (2-inch) heavy copper tubing which had been chromium-plated. Knurled and threaded caps are used at each end to secure rubber gaskets and glass windows made of high grade photographic glass. The vessels are accurately machined to the specified lengths of 3, 6, and 9 cm. The 9-cm. vessel is ordinarily used for white sugar solutions, while the other absorption cells are used for light colored sugar liquors.

Operation of Instrument. The operation of the instrument is simple and can be performed by nontechnically trained analysts. First, the instrument light switch is turned on, the two slide-wires are set at zero, and the instrument is balanced with the blue filters in place and then with the infrared filters in place, simply by moving the appropriate filters into the light path and adjusting the respective variable resistances. The absorption vessel filled with the 50% white sugar solution is next placed in the light path to the measuring photocell. The slide-wire for the blue filters is then rotated until a condition of balance is obtained, and the procedure is repeated with the infrared filters in place, using the second slide-wire for the latter filters. The reading with infrared filters is then subtracted from the reading with the blue filters to give the color of the sample directly. In order to express colors on a 100% solids basis, the color reading of the 50% sugar solution is multiplied by 2.

Calibration of Instrument. The two slide-wire scales are calibrated directly in per cent absorption, each one covering the range from 0 to 50% absorption. Thus, it is a simple matter to provide a second calibration scale indicative of $-\log T$ for the particular wave length involved. This was done for the slide-wire used with the blue filters and the reading is designated as $-\log T_B$. Obviously, however, the same response was not obtained with the infrared filters and it was therefore necessary either to calibrate the other slide-wire in terms of $-\log T$ at the wave length of the infrared filters and correct it later by conversion to a $-\log T_B$ basis, or to incorporate the conversion directly in the calibration. For convenience and simplicity, the latter method was used. However, it was first necessary to determine the relation between the $-\log T_B$ readings and the $-\log T_{IR}$ readings.

In order to ascertain this relation, several series of specially prepared sugar solutions were prepared. These solutions consisted of a specially treated clear, colorless white sugar solution to which known increments of stock color solution and stock turbid solution were added. The stock color solution was prepared by clarifying a colored sugar solution, and the stock turbid solution was prepared by adding bentonite to a colorless sugar solution.

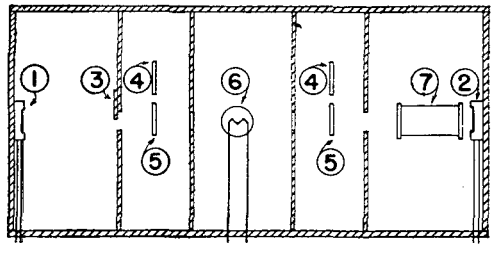

Figure 4. Optical Arrangement of Photoelectric Colorimeter for White Sugar Solutions

1. Comparison photocell
2. Measuring photocell
3. Adjustable aperture
4. Infrared color filters
5. Blue color filters
6. Projection lamp
7. Absorption vessel

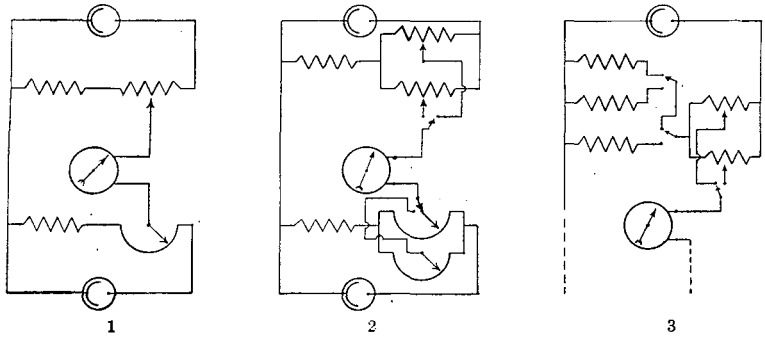

Figure 5. Development of Photoelectric Circuit

1. Basic circuit (one color filter)
2. Combination circuit (two color filters)
3. Modified circuit (showing compensating features)

Seven series of such samples, covering a range of from 0 to about 1500 C and H color units, were prepared and some 150 individual solutions were measured on the instrument, using both the blue and infrared filters. The results of these tests are summarized in Table I. The average ratio of $-\log T_B$ to $-\log T_{IR}$ for any particular turbidity increment is 2.0. This means that any reading with the infrared filters has to be multiplied by 2 in order to provide a correction that can be subtracted directly from the reading with the blue filters. For example, if, with a given colorless sample, a reading of 0.020 is obtained with infrared light, then for the same sample the reading will be 0.040 with blue light. Therefore, the turbidity correction to be applied to the blue reading is 0.040 and not 0.020. The $-\log T$ scale on the slide-wire associated with the infrared filters was calibrated to include this factor of 2.0. Thus the blue slide-wire was equipped with a scale reading directly in $-\log T$, while the infrared slide-wire was equipped with a scale reading $2.0 \times (-\log T)$. This simple adjustment made the subtractive turbidity correction possible. In this particular case, T represents transmission through the 9-cm. absorption cell. Obviously the values can be converted to a 1-cm. thickness basis.

Table I. Tests on Special Samples Containing Varying Increments of Color and Turbidity

(Corning No. 554 Lantern blue and Wratten No. 88A infrared filters)

Test No.	No. of Samples in Test Series	Average Turbidity Increments: Blue filter ($-\log T$)	Infrared filter ($-\log T$)	Ratio B/IR
1	10	0.018	0.0095	1.90
2	25	0.0145	0.0072	2.02
3	24	0.108	0.0555	1.95
4	23	0.0222	0.0110	2.02
5	25	0.0548	0.0266	2.06
6	25	0.0266	0.0119	2.23
7	25	0.046	0.0225	2.04
			Av.	2.0

Because $-\log T$ units are fundamental in nature, all investigative work was expressed in these units. However, for practical purposes, the C and H color units (*11*) previously mentioned were retained for routine color determinations and the slide-wires calibrated to read in these units. This color scale is proportional to a $-\log T$ scale and conversion from one scale to the other may be accomplished by the use of the proper factor. In order to determine this factor as precisely as possible, a long series of color comparisons was made between visual determinations and photoelectric measurements. The relationship so obtained from the average of these many comparisons indicated that multiplying $-\log T$ values obtained on the instrument by a factor of 2800 would give C and H color of 50% sugar solutions. To convert directly to 100% solids, a factor of 5600 can be used.

DISCUSSION OF RESULTS

The development of this photoelectric compensating colorimeter for determination of color of white sugar solutions has made possible a very distinct improvement in the color determinations made in this laboratory. Table II is a comparison of results by four observers on five different samples; color determinations were made both visually and photoelectrically. These observers were experienced in the use of the visual method but were not particularly well acquainted with the photoelectric instrument. In the case of the visual observations, the average spread for the samples was approximately 140 C and H color units, whereas for the photoelectric determinations on the same samples, the average spread was only about 30 C and H color units.

In addition to making available a much more accurate and reproducible method of determining color, this instrument also provides a less exacting procedure by which a nontechnically trained laboratory assistant may secure reliable and reproducible results, thus replacing the highly trained technical personnel previously required for color determinations. Obviously, this has had a tremendous bearing on plant and laboratory investigations in which color has played a major role.

This instrument is also used to determine the color of the light-colored sugar liquors in the refinery. These liquors contain slightly more color and turbidity than the solutions of white sugars and consequently can be more advantageously read with the 6-cm. absorption vessel.

Although the instrument was primarily developed to secure a more accurate measure of color, a measure of relative turbidity is obtained simultaneously. The turbidity results obtained with this instrument have been compared with photoelectric turbidity readings made on another photoelectric device which was developed earlier for measuring the Tyndall effect in sugar solutions (*2*). These comparative readings have shown a constant relationship, indicating that the new instrument can be used to provide a reliable index of turbidity, as well as color.

SUMMARY

An investigation was made of methods of measuring colors of white sugar solutions; these had previously been determined in this laboratory by a visual comparison method. The visual results were not sufficiently accurate or reproducible for control or investigative purposes. An investigation resulted in the development of a compensating photoelectric colorimeter, which provided a means of correcting for the turbidity in the solutions.

In this colorimeter two different filters are employed. Measurements with the Corning No. 554 Lantern blue glass filter are representative of the total absorption of the sugar solutions—i.e., color plus turbidity. Readings with the Eastman Wratten No. 88A infrared filter are indicative of turbidity alone. The two instrument scales have been placed on a common basis, so that color values compensated for turbidity may be obtained from the instrument simply by subtracting the reading with the infrared filter from that with the blue filter. A number of improvements have been made in the design of this instrument to facilitate its operation.

This colorimeter has been in use in this laboratory for several years and has given very satisfactory service. Its adoption has greatly simplified color measurements and permitted the use of nontechnical personnel for such work. The results obtained are much more accurate than those obtained with previous methods and the instrument has thus provided a much more reliable method for control and investigations involving the color of white sugar solutions.

The development of this instrument, together with the previous photoelectric devices, has entirely eliminated the use

Table II. Comparison of Photoelectric and Visual Color Methods

(C and H color units)

Sample	Sugar Solution: Confectioners', %	Sugar Solution: Granulated, %	Visual Color: Observer A	B	C	D	Average	Max. deviation between observers	Photoelectric Color: Observer A	B	C	D	Average	Max. deviation between observers	Difference between Av. Visual and Av. Photoelectric Color
1	100	0	190	170	95	240	175	145	190	195	205	190	195	15	20
2	75	25	340	350	270	380	335	110	340	330	350	310	335	40	0
3	50	50	470	470	450	520	480	70	470	450	470	480	470	30	10
4	25	75	590	620	610	770	650	180	600	630	630	640	625	40	25
5	0	100	810	710	800	910	810	200	760	760	770	750	760	20	50
							Av.	140						30	

of visual methods of color determination in this laboratory. This phase of the work has been completed. However, further investigations are in progress on the development of instruments for measuring the colors of dry white sugars and for photoelectrically indicating the color of refinery liquors on a continuous flow basis.

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Ammonium Hexanitratocerate as a Primary Standard in Oxidimetry

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Ammonium hexanitratocerate (under the name hexanitrato ammonium cerate, now abandoned) has been described (5) as "a proposed reference standard in oxidimetry." The present paper presents experimental data in proof of the fact that ammonium hexanitratocerate may now be accepted as an unqualified primary standard reagent. Its use is also standard for the preparation of evaluated Ce(IV) solutions. The process involves a direct weighing, followed by solution in either perchloric or sulfuric acid and dilution to suitable known volumes. An additional precision process in its use consists in the simple preparation from weighed quantities of ammonium hexanitratocerate (by precipitation using excess ammonium hydroxide) of ceric hydroxide, $Ce(OH)_4$, or hydrous ceric oxide, $CeO_2.2H_2O$, in accurately known amounts. The precipitated product thus obtained is easily soluble in warm dilute sulfuric acid. This process makes practical the preparation of standard sulfatoceric acid [$H_4Ce(SO_4)_4$] solutions free from ammonium and nitrate ions whose presence might conceivably be objectionable. It thus serves as a substitute for a corresponding ammonium sulfatocerate with primary standard purity, a reagent that has not yet been prepared.

A SUMMARY of work dealing with the use of Ce(IV) in volumetric analysis complete to 1942 has been provided (2). No other Ce(IV) salt has been suggested as a primary standard for use in the preparation of accurately standardized volumetric cerate solutions.

Ammonium hexanitratocerate [$(NH_4)_2Ce(NO_3)_6$] complies with practically all desirable properties of a primary standard chemical. It is a stock item from all standard supply sources in primary standard purity. The reagent grade purity is also manufactured. It is an anhydrous salt stable in air under ordinary conditions and at 85° C. for extended and adequate periods of time. It has an exceptionally high equivalent weight (548.26) and but one transferred electron is involved in the change from Ce(IV) to Ce(III). It is instantly and speedily reactive in acid solution under readily attained conditions with solutions of the most reliable primary standards such as arsenious oxide, and sodium oxalate or the less desirable primary standard Mohr's salt, $(NH_4)_2SO_4.FeSO_4.6H_2O$. No possible intermediate valence stages are involved in its use as an oxidant. A large number of internal redox indicators are applicable in the determination of the precise equivalent quantities of reductants with which it reacts. Ammonium hexanitratocerate is easily tested chemically for purity. Stable standard solutions are readily prepared from it by direct weighing, solution in suitable media, and dilution to definite volume, and are of wide applicability. In this respect it rivals potassium permanganate, the use of which it is rapidly supplanting in a great many commercially important analytical control and research practices.

PREPARATION OF PRIMARY STANDARD AMMONIUM HEXANITRATOCERATE

The preparation of chemically pure ammonium hexanitratocerate has been described (5), and the same preparational technique and an alternate procedure were applied in the present work. Two separate samples of the reagent, supplied through the courtesy of The G. Frederick Smith Chemical Company, represented a portion of two 50-pound batches prepared for commercial distribution at widely separated time intervals to be used as primary standard chemicals. They were analyzed as received without further treatment except for drying a minimum of 1 hour at 85° C. This makes certain the elimination of any trace of adsorbed moisture. The drying temperature employed would not make certain the removal of any occluded moisture. The results of the analyses, however, made certain the practically complete absence of occluded moisture within the crystal lattice. This obviates the necessity for drying the product at a higher temperature than that required for the removal of adsorbed moisture.

In an alternative preparational procedure dilute nitric acid (1 volume of nitric acid, specific gravity 1.42, diluted to 4 volumes with water) may be substituted for boiling concentrated nitric acid (5) in the purification of ammonium nitratocerate with equally good results.

Table I. Stability of Ammonium Hexanitratocerate at Moderate Drying Temperatures

Sample No.	Sample Weight	Temperature	Time of Heating	Total Weight Loss	Weight Loss
	Grams	° *C.*	*Hours*	*Mg.*	%
1	3.1973	79	2	10.5	0.033
			4	No loss	0.033
			6	No loss	0.033
			8	No loss	0.033
			10	No loss	0.033
			24	No loss	0.033
2	1.6093	87	2	0.5	0.031
			4	1.0	0.062
			6	No loss	0.062
			8	No loss	0.062
			10	No loss	0.062
			24	1.2	0.074
3	1.4259	99	2	0.2	0.014
			4	0.7	0.042
			6	1.2	0.084
			8	2.1	0.147
			12	3.0	0.210
			28	3.3	0.252

PRACTICAL DETERMINATION OF CASUAL IMPURITIES IN AMMONIUM HEXANITRATOCERATE

Simple tests should prove of value in the preliminary examination of a supposedly pure sample of ammonium hexanitratocerate. The water-solubility test should show no insoluble matter from a 0.01-mole sample.

Dissolve 5.5 grams of the salt in a 400-ml. beaker, using the least required amount of distilled water at room temperature, and stirring vigorously to complete solution of the sample. Under these conditions (a practically saturated solution of the salt) there is no resultant hydrolysis, as such solutions have a pH of approximately 1. The formation of soluble hydrolytic products with accompanying liberation of free nitric acid (*2*) accounts for this condition. The solution thus prepared should be sparkling clear, red in color, and entirely free from insoluble material such as ceric oxide. This qualitative test would indicate definitely that the reagent had not been dried at a higher temperature than that permissible—namely, 85° C.

Iron as impurity is the only heavy metal expected. Apply the iron test to the saturated solution of the sample used in the determination just described. Add dropwise with constant stirring a 30% solution of hydrogen peroxide until the orange color is almost removed. Complete the reduction of Ce(IV) by the dropwise addition of 3% hydrogen peroxide. Add 500 mg. of hydroxylamine hydrochloride to reduce the iron. Neutralize the resulting solution by the dropwise addition of dilute ammonium hydroxide until Congo red paper changes in color from blue to red. Add 5 ml. of a saturated aqueous solution of 1,10-phenanthroline to complex the ferrous ion and produce the characteristic red color from any iron present (*4*). Carry out an accompanying blank test on the reagents employed, but omit the sample of cerium salt. Dilute both test solutions to 250 ml. and examine for color due to iron. An excess of 2 p.p.m. of iron as impurity should not be indicated, as shown by comparison with a standard color prepared from a known amount or iron similarly treated.

The process of manufacture previously described (*5*) gives a finished product which by repeated spectroscopic examination has been shown to be free from thorium and other cerium-associated rare metal impurities. These spectroscopic tests have been applied both by the authors and by independent investigators at the University of Chicago.

The following analysis is indicative of purity by determination of the ignition residue.

Place an accurately weighed, approximately 1-gram sample of the salt in an ignited and accurately weighed porcelain crucible, and decompose the sample by gradually heating the crucible over a very low flame. This process will leave a residue of ceric oxide which upon subsequent ignition to constant weight at 900° C. constitutes 31.396% of the original sample. Failure to meet this test within reasonable experimental error indicates a correspondingly mpure product.

A final simple solubility test indicates purity to a marked degree. A 1-gram sample should be completely soluble in 75 ml. of absolute ethyl alcohol. *If 95% alcohol is employed,* the test is less valuable and the color of the resulting solution much more intense. This solubility test is more stringent than the water-solubility test, because of the greatly diminished solubility of possible impurities in absolute alcohol.

REAGENTS EMPLOYED

Ammonium Hexanitratocerate. A G. Frederick Smith Chemical Company primary standard grade with samples from 50-pound batches prepared for distribution through trade channels. The samples were dried for 4 to 6 hours at 85° to 87° C. at the time of sampling.

Arsenious Oxide. National Bureau of Standards sample 83a. Certificate value, 99.99% purity.

Sodium Oxalate. National Bureau of Standards sample 40e. Certificate value, 99.96% purity.

1,10-Phenanthroline Ferrous Sulfate, 0.025 *M* solution (ferroin).

5-Nitro-1,10-phenanthroline Ferrous Sulfate, 0.025 *M* solution (nitroferroin).

Water. Conductivity water only was employed for the preparation of all solutions and for all titrations and precipitations.

Perchloric acid, C.P. vacuum distilled 72% acid manufactured by The G. Frederick Smith Chemical Company.

Osmic Acid Catalyst, 0.01 *M* solution in 0.1 *N* sulfuric acid.

STABILITY OF AMMONIUM HEXANITRATOCERATE AT VARIOUS DRYING TEMPERATURES

In the original description of ammonium hexanitratocerate as a "proposed" primary standard reagent a drying temperature of 110° C. was specified (*5*). The drying of small samples for periods of 1 hour at this temperature introduces but a minor error, but if the drying time at 110° C. is extended to larger time intervals the error becomes appreciable. Accordingly, stability tests were applied at various temperatures to re-establish a correct drying temperature (Table I).

By examination of the data of Table I the highest determined permissible drying temperature was found to be 87° C. At 99° there is a small but progressively cumulative loss in weight. Sample 1 was dried as received. Samples 2 and 3 were dried at 79° preliminary to the drying experiments recorded in Table I. The samples employed in all the remaining analyses in this work were dried for 4 to 6 hours at 80° to 85° C., followed by storage in desiccators over Anhydrone.

OUTLINE OF ANALYTICAL PROCEDURES

A detailed description of the titrational methods employed for the evaluation of the ammonium nitratocerate is given in (*2*, Chapter 4).

Method A. Weighed samples of ammonium hexanitratrocerate were dissolved in perchloric acid and titrated, using a solution of accurately known concentration of sodium oxalate dissolved in perchloric acid. For these titrations nitroferroin (5-nitro-1,10-phenanthroline ferrous sulfate) served as indicator.

Method B. Weighed samples of the same salt were dissolved under the same conditions and titrated, using a solution of accurately known concentration of arsenious acid made from known weights of Bureau of Standards arsenious oxide, also in perchloric acid solution. For this titration a drop of 0.01 *M* osmic acid in 0.1 *N* sulfuric acid solution was employed as catalyst. Nitroferroin again served as indicator.

Method C. Weighed samples of the same material were dissolved in a small amount of water and added to a twofold excess of dilute ammonium hydroxide in the apparatus shown in Figure 1. The ceric hydroxide thus quantitatively precipitated was filtered and washed with five or six portions of water to remove ammonium nitrate. Finally, the washing ceric hydroxide was dissolved in hot 2 *F* sulfuric acid and the contents of the reaction vessel were withdrawn into a 300-ml. filter flask. The reaction flask and filter disk were thoroughly washed with additions of sulfuric acid and the sulfatoceric acid thus obtained was titrated using standard arsenite prepared as for Method B, with osmic acid as catalyst but with ferroin in place of nitroferroin as indicator. [There are two sulfatoceric acids known to exist, having

the formulas $H_2Ce(SO_4)_3$ and $H_4Ce(SO_4)_4$. The ammonium salt of formula $(NH_4)_2Ce(SO_4)_3$ may be precipitated from solutions of the former by the addition of ammonium sulfate to solutions of the acid which have only sufficient free sulfuric acid present to prevent hydrolysis. The latter sulfatoceric acid, when present with higher concentrations of sulfuric acid, upon similar treatment forms $(NH_4)_4Ce(SO_4)_4.2H_2O$. This tetraammonium sulfatocerate is often described as a double salt, $2(NH_4)_2SO_4.Ce(SO_4)_2.2H_2O$, and is known as Coffelt's salt.]

The reactions involved are as follows:

Method A

$$2Ce(NO_3)_6^{--} + C_2O_4^{--} + [HClO_4] = 2Ce^{+++} + 2CO_2 + 12NO_3^-$$

$$Ce(NO_3)_6^{--} + [(C_{12}H_7N_3O_3)_3Fe]^{++} + [HClO_4] = Ce^{+++} + [(C_{12}H_7N_3O_3)_3Fe]^{+++} + 6NO_3^-$$

Method B

$$2Ce(NO_3)_6^{--} + AsO_3^{---} + [HClO_4] + [\text{Osmic Acid}] + H_2O = 2Ce^{+++} + AsO_4^{---} + 2H^+ + 12NO_3^-$$

(Indicator reaction the same as for Method A)

Method C

$$Ce(NO_3)_6^{--} + 4NH_4OH = Ce(OH)_4 + 4NH_4^+ + 6NO_3^-$$

$$Ce(OH)_4 + 4H_2SO_4 = [Ce(SO_4)_4]^{----} + 4H_2O + 4H^+$$

$$2Ce(SO_4)_4^{----} + AsO_3^{---} + H_2O + [H_2SO_4] + [\text{Osmic Acid}] = 2Ce^{+++} + AsO_4^{----} + 2H^+ + 8SO_4^{--}$$

$$Ce(SO_4)_4^{----} + [(C_{12}H_8N_2)_3Fe]^{++} + [H_2SO_4] = Ce^{+++} + [(C_{12}H_8N_2)_3Fe]^{+++} + 4SO_4^{--}$$

PREPARATION OF PRIMARY STANDARD ARSENITE AND OXALATE SOLUTIONS

Weighed quantities of sodium oxalate (National Bureau of Standards No. 40e) were dissolved in 2 *F* perchloric acid, transferred to a weighed 300- or 600-ml. glass-stoppered flask, and diluted to approximately 300 to 600 ml., employing 2 *F* perchloric acid. For weighings under 100 grams, a Troemner No. 10 balance was employed together with a calibrated set of standard weights. The solution weights were taken using an August Sauter balance of 10-kg. capacity with a sensitivity of 10 mg. or better. Calibrated weights were also employed in using this balance. Buoyancy corrections were applied in all weighings. The standard solutions thus prepared were used to titrate weighed portions of the nitratocerate; in all titrations weight burets of design previously described (*3*) were employed. Weight buret readings were determined after applying buoyancy corrections using the Troemner balance.

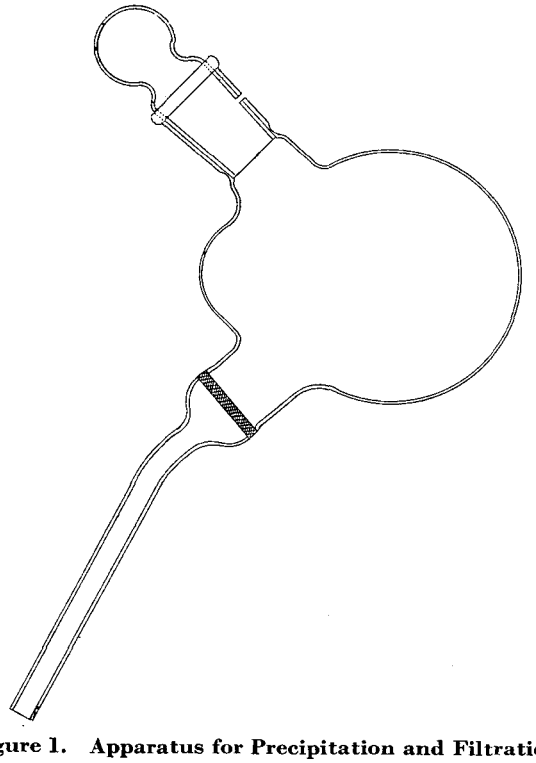

Figure 1. Apparatus for Precipitation and Filtration

Table II. Preparation of Arsenite and Oxalate Solutions for Evaluating Purity of Ammonium Hexanitratocerate

Solution No.	Type	$Na_2C_2O_4$ or As_2O_3 Taken *Grams*	Solution Weight *Grams*	Acid Used	Weight of Salt *G./g.*	Calcd. Value of $(NH_4)_2Ce(NO_3)_6$ *G./g.*
1	Arsenite	3.0019(5)	1016.72(9)	2 *F* $HClO_4$	0.0028274	0.031345
2	Arsenite	3.0103(0)	1055.50(6)	1 *F* H_2SO_4	0.0028520	0.031611
3	Oxalate	3.2335(0)	548.37(6)	2 *F* $HClO_4$	0.0060606	0.049450
4	Arsenite	2.2845(0)	516.32(1)	2 *F* $HClO_4$	0.0044245	0.049068
5	Oxalate	1.5409(5)	278.58(4)	2 *F* $HClO_4$	0.0055313	0.045259
6	Arsenite	2.2818(5)	601.34(1)	1 *F* H_2SO_4	0.0038112	0.045251

Sodium oxalate or arsenious oxide (equivalent weights roughly 67 and 49.5, respectively), because of their low values, make preferable the titration of weighed samples of ammonium hexanitratocerate by oxalate or arsenite to the reverse titration. By use of 0.05 to 0.06 *N* strength of titrating solution a 40-gram sample reacts with 1.1 to 1.3 grams of ammonium hexanitratocerate. Such samples, individually weighed, afford better accuracy than the weighing of 0.2 to 0.25 gram of arsenious oxide or sodium oxalate which would be required for individual titrations by the reverse procedure. An added advantage, following the procedure as chosen, consists in the fact that solutions of ammonium nitratocerate acidified using perchloric acid change titer detectably in 72 to 96 hours, whereas the solutions of arsenite or oxalate containing perchloric or sulfuric acid are stable indefinitely. The potentiometric titrational characteristics of these reactions have been described (*2*, sections 3 and 4).

Solutions of predetermined strength in arsenious oxide were prepared from National Bureau of Standards primary standard sample 83a with acidification using either 2 *F* perchloric acid or 1 *F* sulfuric acid as solvent. The weighed portions were placed in 250-ml. beakers and dissolved in a small volume of water by the addition of sufficient pellet sodium hydroxide to form sodium arsenite. These samples were then acidified with acids of the proper strength and transferred to 500- or 1000-ml. glass-stoppered flasks and the solutions were weighed as previously described.

The data governing the preparation of the arsenite and oxalate solutions are given in Table II.

DETERMINATION OF PURITY OF AMMONIUM HEXANITRATOCERATE SAMPLE 1

Sample 1 of ammonium hexanitratocerate was analyzed by solution in perchloric acid and titration, using standard of reference arsenious oxide and sodium oxalate as two individual and distinctive procedures. The third, and radically altered, procedure followed the scheme of the precipitation of Ce(IV) from excess ammonia solution, filtration, washing, solution in sulfuric acid, and final titration by a sulfuric acid solution of sodium arsenite prepared from primary standard arsenious oxide. Standard solutions 1, 2, and 3 (prepared as shown in Table II) were employed for these analyses. The results of the first two procedures agree to a very close tolerance (by the two directly applied analyses). The results of the indirect ceric hydroxide isolation, solution, and titration [an indirect procedure involving isolation of Ce(IV)] agree satisfactorily with the direct titrations and serve to "make assurance doubly sure." This indirect evaluation

Table III. Determination of Purity of Ammonium Hexanitratocerate Sample 1

(Employing As_2O_3 and $Na_2C_2O_4$ as primary standards)

$(NH_4)_2Ce(NO_3)_6$ Sample Weight	Reference Solution (Table II)	Weight of Standard Soln. Required	$(NH_4)_2Ce(NO_3)_6$ Indicated	Error of Recovery	Calcd. Salt Purity
Grams		*Grams*	*Grams*	*Mg.*	%
1.3024(3)	1	41.546	1.3022(4)	−0.19	99.97(9)
1.3039(8)	1	41.597(5)	1.3038(7)	−0.11	99.98(4)
1.3055(3)	1	41.651	1.3055(4)	+0.01	100.00
1.3043(8)	1	41.609	1.3042(2)	−0.16	99.98(3)
				Av.	99.985
1.0066(8)	3	20.297(5)	1.0065(5)	−0.13	99.98(7)
1.0076(8)	3	20.328(5)	1.0080(8)	+0.40	100.03(7)
1.0050(7)	3	20.266(5)	1.0050(2)	−0.05	99.99(5)
1.0270(0)	3	20.713(5)	1.0271(7)	+0.17	100.01(6)
1.0093(8)	3	20.351	1.0092(1)	−0.17	99.98(3)
1.0051(7)	3	20.267(5)	1.0050(6)	−0.11	99.98(9)
				Av.	100.00(1)
1.3016(1)	2	41.164	1.3012(4)	−0.37	99.97(5)
1.3035(1)	2	41.229	1.3032(9)	−0.22	99.98(8)
1.3048(1)	2	41.262	1.304(4)	−0.47	99.96(6)
1.3045(1)	2	41.252	1.3040(1)	−0.50	99.97(1)
				Av.	99.97(5)

Av. of 14 individual analyses, 99.98(9)% purity

Table IV. Determination of Purity of Ammonium Hexanitratocerate Sample 2

(Employing As_2O_3 and $Na_2C_2O_4$ as primary standards)

$(NH_4)_2Ce(NO_3)_6$ Sample Weight	Reference Solution (Table II)	Weight of Standard Soln. Required	$(NH_4)_2Ce(NO_3)_6$ Indicated	Error of Recovery	Calcd. Salt Purity
Grams		*Grams*	*Grams*	*Mg.*	%
1.0039(3)	4	20.345(5)	1.0038(5)	−0.08	99.99(2)
1.0022(3)	4	20.307	1.0019(6)	−0.27	99.97(3)
1.0076(8)	4	20.397	1.0063(8)	−0.40	99.96(0)
1.0021(3)	4	20.311	1.0021(3)	±0.00	100.00
1.0063(8)	4	20.395	1.0062(8)	−0.01	99.99
1.0074(3)	4	20.406(5)	1.0068(6)	−0.50	99.97(9)
				Av.	99.97(7)
1.0022(1)	5	22.140	1.0020(2)	−0.19	99.98(1)
1.0045(3)	5	22.189	1.0042(5)	−0.28	99.97(1)
1.0204(5)	5	22.541	1.0201(8)	−0.27	99.97(4)
1.0133(3)	5	22.387	1.0132(1)	−0.12	99.98(8)
				Av.	99.97(9)
1.094(1)	6	26.251(5)	1.1091(5)	−0.26	99.97(6)
1.0795(3)	6	25.539	1.0790(4)	−0.49	99.95(5)
1.0867(7)	6	25.711(5)	1.0863(4)	−0.43	99.96(1)
1.0496(6)	6	24.841(5)	1.0495(1)	−0.15	99.99(1)
1.0379(6)	6	24.561(5)	1.0377(4)	−0.22	99.97(8)
1.0846(1)	6	25.662	1.0842(3)	−0.38	99.96(5)
				Av.	99.96(9)

Av. of 16 individual analyses, 99.97(7)%

of the oxidation equivalent of the hexanitratocerate proves conclusively that its total oxidation value lies in its cerium content. In addition, the procedure as described is shown to be a reliable scheme for the preparation of solutions of sulfatoceric acid [$H_4Ce(SO_4)_4$] of predetermined titer without the ammonium nitrate and nitric acid which would be present if such solutions were to be prepared by direct solution of ammonium hexanitratocerate in sulfuric acid and dilution to a fixed volume. The data are given in Table III. Buoyancy corrections were calculated using the determined value of 2.61 as the specific gravity of ammonium hexanitratocerate at 25° C.

By examination of Table III it is observed that the maximum average deviation in results among the three methods is 2 parts in 10,000. The average analysis in 14 determinations indicates that the purity of this sample of hexanitratocerate is 99.99%. The indirect procedure of analysis, following isolation of this salt's cerium content, shows results 0.01% lower—namely, 99.98%. This lower value may be attributed to the introduction of additional manipulative processes of a less direct nature, following which the values were determined. The results given in Table III are consecutive analyses with no omission in any given system of analyses. The recommended purity given with the Bureau of Standards certificates of 99.99% for standard 83a arsenious oxide and 99.96% for sodium oxalate sample 40e were applied to the calculations of Table III. Solution densities for buoyancy correction were calculated from weight data and the specific gravity of the acids used in their preparation.

The procedures described in the analysis of sample 1 of ammonium hexanitratocerate were repeated in the case of sample 2 with results shown in Table IV.

By examination of Table IV, the same conclusions may be drawn as those observed with regard to Table III. The most probable value for the purity of ammonium nitratocerate sample 2 is 99.98%.

ERRORS IN CONVERSION OF AMMONIUM HEXANITRATOCERATE TO PERCHLORATOCERIC ACID AND CERIC HYDROXIDES TO SULFATOCERIC ACID

Solutions of ammonium hexanitratocerate in 1 *F* perchloric acid have an oxidation potential of 1.71 volts; they are not permanently stable and very gradually diminish in oxidation value with time of storage. If stored at ice box temperatures and in dark bottles, solutions (even of 0.001 *N* concentrations) need be restandardized only at 72- to 96-hour intervals (*1*). For the preparation of 0.05 to 0.1 *N* solutions of ammonium hexanitratocerate in perchloric acid for use in macrovolumetric determinations, restandardization is required only after 72 hours' storage (*2*). Storage in dark bottles in a cool place is recommended.

In making perchloric acid solutions of ammonium hexanitratocerate, great care should be taken to avoid the presence of organic matter. The importance of pure water and thoroughly clean containers cannot be overemphasized. Cellulose, sugars, alcohols, and many other organic materials are readily and quantitatively oxidized by the cerate ion in perchloric acid solution (*2*). The use of too much stopcock grease in burets may introduce errors. The presence of carbonaceous dusts causes perchloratocerate solutions to change titer. Contact with platinum in the form of sponge or platinum black causes comparatively rapid reduction of such solutions. These are all penalties associated with an oxidation medium of exceptionally high oxidation potential.

Special directions must be followed in the preparation of solutions of ammonium hexanitratocerate in perchloric acid.

To prepare such a solution of 0.1 *N* strength, 54.826 grams (corrected weight of the pure salt) are placed in a 1000-ml. beaker and 83.8 ml. of 72% perchloric acid are added. The salt is not soluble in perchloric acid of this strength, but should be stirred with it for 2 minutes. A 100-ml. portion of water is added and the stirring is continued for 2 minutes. The addition of 100 ml. of water is repeated a third, fourth, and fifth time with intermediate stirring for 2-minute intervals. By this necessary procedure all the ammonium hexanitratocerate will be in solution at 500- to 600-ml. volume and may be transferred to a 1000-ml. graduated flask and diluted to volume. The ammonium hexanitratocerate would dissolve completely to form a crystal-clear solution if it were added to the perchloric acid after dilution with water to 500 to 600 ml. Such a solution after 24 hours would be partially precipitated, owing to the presence of complex nitratoperchloratocerates which are sparingly soluble. If the salt crystals are added to 72% perchloric acid the $Ce(NO_3)_6^{--}$ complex ion is sufficiently completely converted to the $Ce(ClO_4)_6^{--}$ ion to prevent the formation of insoluble material.

The substitution of sulfuric acid for perchloric acid requires that a duplicate solution procedure be applied with slow addition of the first 100 ml. of water over a 5-minute period with stirring

(56 ml. of 95% sulfuric required in place of 83.8 ml. of 72% perchloric acid). Mixed nitratosulfatocerates are more insoluble than the mixed nitratoperchloratocerates and would precipitate copiously upon standing were this procedure not followed.

Solutions of sulfatoceric acid, $H_4Ce(SO_4)_4$, and perchloratoceric acid, $H_2Ce(ClO_4)_6$, prepared by the process described above, contain ammonium and nitrate ions. The solutions are of known normality and for most applications serve admirably (*2*). If ammonium and nitrate ions are undesirable, solutions of perchloratoceric acid are best prepared by the electro-oxidation of perchloric acid (*2*, pages 5 to 9). For the preparation of sulfatoceric acid the method described above in connection with the determination of the purity of ammonium hexanitratocerate is employed. The apparatus in Figure 1 serves conveniently for the precipitation of ceric hydroxide, with removal of the ammonium and nitrate ions, solution of the ceric hydroxide in warm 2 *F* to 4 *F* sulfuric acid, and final dilution to the predetermined volume. An Ace sintered-glass filtering crucible with porosity E may be used to filter and wash ceric hydroxide. Filter paper or a platinum Munroe filtering crucible must be avoided. If filter paper is used some of the sulfatoceric acid is invariably reduced; results may be 1 to 2% low. Low results are also caused by contact between hot sulfatoceric acid and platinum sponge if a Munroe crucible is employed.

DECOMPOSITION OF PERCHLORATOCERIC ACID SOLUTIONS IN CONTACT WITH PLATINUM SPONGE

A solution of approximately 0.1 *N* perchloratoceric acid was prepared from ammonium hexanitratocerate and perchloric acid, and was stored in contact with platinum sponge formed by the ignition of 1 gram of chloroplatinic acid. The perchloratoceric acid solution was stirred continuously at ordinary temperatures and analyzed at stated intervals. The original normality was 0.1020. After 2 hours it was 0.0996, after 5 hours 0.0980, after 22 hours 0.0925, and after 96 hours 0.0775 *N*. This is a drop of almost 25% in oxidation value. Sulfatocerate solutions are not as extensively affected, but the loss is readily noted when hot solutions are filtered using a platinum Munroe filtering crucible. Graphitic carbon is known to exert a similar catalytic reducing effect.

Table V. Analysis of Sample 1 after More Than 2 Years' Storage under Ordinary Conditions

[Test of stability and retention of oxidation value with long-term storage. As_2O_3 solution, 0.027415 gram per gram, equivalent to 0.303935 gram of $(NH_4)_2Ce(NO_3)_6$ per gram of solution]

Sample No.	As_2O_3 Soln.	$(NH_4)_2Ce(NO_3)_6$ Taken	$(NH_4)_2Ce(NO_3)_6$ Found	Purity
	Grams	*Grams*	*Grams*	%
1	10.3884(6)	3.1578(4)	3.1573(5)	99.986
2	8.5872(4)	2.6101(0)	2.6099(6)	99.994
3	8.1556(2)	2.4788(4)	2.4787(8)	99.997
4	8.3569(5)	2.5402(5)	2.5399(7)	99.989
5	7.8023(9)	2.3719(6)	2.3714(2)	99.977
6	8.9750(8)	2.7281(2)	2.7278(9)	99.990
			Av.	99.988

STABILITY OF CRYSTALLINE AMMONIUM NITRATE WITH LONG PERIODS OF STORAGE

Ammonium nitratocerate consists of oxidizable cations, $2NH_4^+$, and a complex anion, $Ce(NO_3)_6^-$, which is known to be a powerful oxidizing material. Solutions of hexanitratoceric acid, $H_2Ce(NO_3)_6$, in nitric acid are known to be unstable to a minute but measurable extent. This instability is brought about by the decomposition of water with the evolution of oxygen through the intermediate liberation of free radical hydroxyl groups. In order to study the shelf life of crystalline ammonium nitratocerate, after completion of the present work and preparation of the manuscript it was thought appropriate to delay 2 years to permit a shelf life test period and subsequent additional analysis. The procedure duplicated that previously described and employed the same corrections and the same drying temperature.

Sample reagent 1 with 2 years and over "shelf existence" was reanalyzed with the results shown in Table V.

Table V shows that the new primary standard, $(NH_4)_2Ce(NO_3)_6$, does not measurably alter in value as an oxidant with extended periods of storage.

SUMMARY

Ammonium hexanitratocerate, proposed as a primary standard in oxidimetry, has been reinvestigated. This salt is commercially available in highly pure form for use as a precision primary standard.

The determination of a satisfactory drying temperature to eliminate adsorbed moisture or nitric acid is shown to be 87° C. in place of the previously recommended temperature of 110°. Occluded moisture is absent from crystalline ammonium nitratocerate, $(NH_4)_2Ce(NO_3)_6$. Ammonium hexanitratocerate is shown to possess the ten most desirable chemical and physical properties governing the selection of a material to be used as a primary standard. Simple qualitative tests are described for use in rapid preliminary testing of samples of ammonium hexanitratocerate for purity.

Two commercially available samples of ammonium hexanitratocerate were standardized by comparison with National Bureau of Standards arsenious oxide (primary standard 83a) and sodium oxalate (primary standard 40e), and their purities were shown to be 99.99 and 99.98%. Their cerium content was isolated as ceric hydroxide and converted to sulfatoceric acid, $H_4Ce(SO_4)_3$. Their purity was thus determined by the use of reference standard arsenious oxide. By this indirect procedure their oxidizing value was shown to be entirely due to the cerium content. The results thus obtained agree with the previous results within 1 part in 10,000.

In the analyses reported all appropriate corrections were applied. The weights were calibrated by comparison with a master set which had been evaluated at the Bureau of Standards. A Troemner No. 10 balance accurate to ±0.05 mg. by direct observation and to ±0.02 mg. by estimation was employed for all weighings of less than 100 grams. A special August Sauter balance of 10-kg. capacity accurate to ±10 mg. was employed for weighing solutions of 250 to 1000 grams. Weight burets were employed for all titrations with corrections for buoyancy applied to all weighings. The weights of arsenious oxide, sodium oxalate, and ammonium hexanitratocerate were likewise corrected to the vacuum basis.

Directions are given for the preparation of sulfatoceric acid from ammonium hexanitratocerate in the form of solutions of predetermined normality. Known amounts of ceric hydroxide are prepared from weighed amounts of the new primary standard, dissolved in hot dilute sulfuric acid, and diluted to a predetermined volume. This process is equivalent to the ability to use a pure sample of ceric oxide as a primary standard. Such samples of pure ceric oxide are not soluble in sulfuric acid.

The shelf life of the new primary standard is adequate.

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Determination of Diglycols in Mixtures of Ethylene and Propylene Glycols

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A method for the determination of diethylene glycol or dipropylene glycol as contaminant in monoglycols is presented. The monoglycols are removed by reaction with periodic acid and distillation. Excess periodic acid is reduced to iodic acid, and the remaining diglycols are oxidized with potassium dichromate. The excess dichromate is measured with a polarograph.

IN THE manufacture of a refined grade of monoglycols having a diglycol content of less than 1%, it becomes necessary to have a rapid and accurate method for the direct determination of the diglycol content. Methods for the estimation of ethylene and propylene glycols (*1–4*) have a precision and accuracy of only 1 to 2%, and, therefore, the diglycol cannot be estimated by difference.

This paper proposes a method for determining diethylene glycol or dipropylene glycol, or both, present in quantities less than 1% in mixtures of ethylene glycol and propylene glycol. The method, however, is not limited to small quantities; diglycols can be determined in a refined grade of monoglycols with an accuracy of ±0.1 to ±0.2% when 0 to 3% diglycols are present. The monoglycols are removed by reaction with periodic acid and distillation. The excess periodic acid is removed by reduction to iodic acid, and the remaining diglycols are oxidized with potassium dichromate. The dichromate is measured with a polarograph before and after the oxidation reaction.

REAGENTS AND EQUIPMENT

Periodic acid, G. F. Smith Chemical Company.
Fuchsin-aldehyde reagent.
Sodium carbonate, C.P. anhydrous.
Hydrogen peroxide, 3% solution.
Sulfuric acid, 1 to 1 aqueous solution.
Potassium dichromate, 0.1 *N*, accurately standardized.
Sodium hydroxide, 1.0 *N*.
Diethylene glycol standard, redistilled and dried β,β-dihydroxyethyl ether, Eastman Kodak No. 2041.
Kjeldahl distilling unit, 250-ml. flask, trap, and vertical condenser.
Reflux condensers with ground-glass connections, and fitted with 500-ml. Erlenmeyer flasks.
Polarograph. The model used in this laboratory is the Sargent-Heyrovský recording polarograph, Model XXI.

PROCEDURE

Weigh and dilute the sample with distilled water so that the aliquot taken for analysis contains about 5 to 10 mg. of diglycols. Transfer the aliquot to a 250-ml. Kjeldahl flask, and add 2 grams of periodic acid dissolved in about 50 ml. of water and a few glass beads. If more than 0.5 gram of monoglycol is present, use a correspondingly larger amount of periodic acid. Dilute with distilled water to about 200 ml. Connect the flask to the distillation unit and distill until the distillate no longer shows an aldehyde reaction with fuchsin-aldehyde reagent. If the residue is reduced to 15 ml. and aldehydes are not removed, add 50 ml. of water to the flask and continue the distillation.

Cool the residue and dilute to 50 ml. Make alkaline with dry sodium carbonate and add 15 ml. of 3% hydrogen peroxide. Distill until the volume of residue is again reduced to about 20 ml. Cool, transfer to a 500-ml. Erlenmeyer flask, and add 100 ml. of 1 to 1 sulfuric acid. If a yellow color appears, boil until it is removed. Add 20 ml. of 0.1 *N* potassium dichromate and reflux 45 minutes. Transfer the cooled refluxed mixture to a 1-liter volumetric flask and dilute to the mark. Transfer a 10-ml. aliquot to a 100-ml. flask and dilute to the mark with 1.0 *N* sodium hydroxide.

Record a polarogram with the instrument set as follows:

D.C. e.m.f.	1.5 volts
Span	1.5 volts
Initial	Zero
Damping	Off
Drop time	Approximately 3 seconds
Dropping mercury electrode	Negative
Sensitivity	0.01 microampere per mm.

Record only the range of 30 to 60% of the voltage applied.

Record a polarogram of 20 ml. of 0.1 *N* potassium dichromate carried through the procedure beginning with the refluxing with sulfuric acid, but without sample added to determine weight of potassium dichromate per microampere recorded. Factor A.

Record a polarogram of a known weight of diglycols beginning with the dichromate oxidation to determine the weight of diglycol equivalent to 1 gram of potassium dichromate. Factor B.

CALCULATION

$$\frac{(\text{Microamperes for blank} - \text{microamperes for sample}) \times \text{factor A} \times \text{factor B} \times 100}{\text{Weight of sample}} = \text{diglycol, \% by weight}$$

ANALYTICAL RESULTS

Experimental samples were prepared containing various amounts of diglycols as contaminants, the major portion of each sample being a mixture of ethylene and propylene glycol in an approximate 60 to 40 ratio. These samples were carried through the procedure outlined above. Some typical results are shown in Table I.

DISCUSSION

In the distillation to remove aldehydes formed from monoglycols, it was noted that the more dilute the aldehyde solution, the more efficiently the aldehydes were removed. If very small

Table I. Typical Analyses of Diglycols as Contaminant in Monoglycols

Contaminant	Contaminant Present %	Contaminant Found %
Diethylene glycol	1.5	1.7
	1.5	1.3
	0.77	0.70
	0.77	0.73
	2.3	2.4
Dipropylene glycol	1.4	1.3
	1.0	1.0
	0.75	0.81
	0.52	0.60
	2.5	2.7
Diethylene glycol plus dipropylene glycol, 60/40 ratio	1.5	1.5
	1.1	1.0
Triethylene glycol	1.3	1.2
	0.8	0.9
None	0	0.05
None	0	−0.1

amounts of diglycols are present, it may be necessary to add water to the residue and redistill to remove the aldehydes completely. This process does not appreciably affect the diglycol determination.

The time specified for refluxing with dichromate is about the minimum time but additional refluxing does not alter results.

An unsuccessful attempt was made to remove the iodine compounds by precipitation as metal salts (*5*, *6*), using lead, barium, bismuth, and other metals so that the dichromate could be titrated with sodium thiosulfate.

For the oxidation of diethylene glycol by potassium dichromate, the following reaction is usually given:

$$3C_4H_{10}O_3 + 10K_2Cr_2O_7 + 40H_2SO_4 \longrightarrow 10K_2SO_4 + 10Cr_2(SO_4)_3 + 12CO_2 + 55H_2O$$

By this reaction, factor B should be 0.1082. This factor, as determined by the above method, was 0.1381 which is roughly equivalent to 8 moles of potassium dichromate to 3 moles of diethylene glycol. This factor is reproducible and was used in this work. The reason for this deviation from the theoretical is not known.

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Determination of Microgram Amounts of Thorium

A Colorimetric Method

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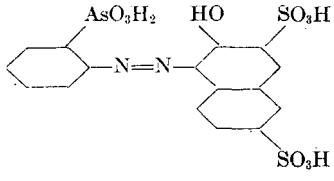
A method for determining thorium in the range 5 to 80 micrograms has been developed, using 1-(*o*-arsonophenylazo)-2-naphthol-3,6-disulfonic acid. Uranium and the rare earths do not interfere in amounts less than 1000 micrograms and interference from iron can be lessened by reduction to the ferrous state.

THE colorimetric determination of thorium on the microgram level has received little consideration in the literature, no doubt because of the absence of characteristic colored complexes of thorium. Survey tests in this laboratory with alizarin and other reagents that are known to complex thorium (*1*, *10*) have not indicated any stable complex that might be used as a basis for a satisfactory colorimetric micromethod. Recently, however, it was reported by Kuznetsov (*7*) that 1-(*o*-arsonophenylazo)-2-naphthol-3,6-disulfonic acid formed a specific red precipitate with thorium in hydrochloric acid. It was stated that by visual comparison 1 microgram of thorium per milliliter could be detected, and a spot test technique was described.



In view of the need for a rapid micromethod for the determination of small amounts of thorium, the reagent was synthesized and the possibilities of developing a spectrophotometric procedure were investigated. As a result, a method has been developed for the determination of thorium in the range of 5 to 80 micrograms per 10 ml. of final volume. The method consists of the following steps: adjustment of pH, addition of the organic reagent, dilution to volume, and measurement of the optical density in a spectrophotometer versus a reference solution of the reagent.

EXPERIMENTAL

Synthesis of Organic Reagent. The 1-(*o*-arsonophenylazo)-2-naphthol-3,6-disulfonic acid was synthesized according to directions given by Kuznetsov.

To 2.17 grams of *o*-aminophenylarsonic acid (0.01 molar) dissolved in 40 ml. of water, add 3 ml. of hydrochloric acid (specific gravity 1.12), cool, and diazotize by adding 5.0 ml. of a 2 *N* solution of sodium nitrite. Filter the diazo compound away from the accidental dirt and rapidly mix with a filtered solution of 4.2 grams of the sodium salt of 2,3,6-naphtholsulfonic acid (R salt) and 4 grams of anhydrous sodium carbonate in 40 ml. of water. The mixture immediately becomes red, and after some seconds a precipitate forms. Let stand for 2 to 3 hours, then slowly warm (the precipitate dissolves) and add 14 grams of clean, solid sodium chloride. On cooling, the mass thickens. Filter, squeeze out the liquid, and wash with cold water. Ruby red crystals are produced. A solution in water and dilute acid is orange colored, in alkali is orange-red, in concentrated sulfuric acid is rose colored. It is not soluble in alcohol.

The required *o*-aminophenylarsonic acid was obtained by reducing *o*-nitrophenylarsonic acid by treatment with ferrous sulfate (better ferrous chloride) and sodium hydroxide (*2–4*). *o*-Nitrophenylarsonic acid is readily prepared by the reaction of Bart by the interaction of diazotized *o*-nitroaniline with an alkaline solution of arsenic trioxide (*5*, *6*, *9*).

By analysis the arsenic content of the recrystallized organic reagent was found to be 13.1%. Theoretical is 13.0% for the disodium salt. A more complete and detailed procedure for the synthesis of the organic reagent is to be published by Reed and associates (*8*).

Materials. Reagent grade chemicals of the common materials were used for testing interferences. Spectrographic analysis of the yttrium nitrate reagent disclosed only the rare earths and calcium as impurities. The zirconyl chloride reagent contained hafnium and traces of iron. No traces of thorium were found in the lanthanum nitrate reagent.

A standard thorium solution was made by dissolving 23.8 grams of chemically pure thorium nitrate tetrahydrate in distilled water and diluting to 1 liter. The thorium nitrate obtained from

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the Lindsay Light and Chemical Company, West Chicago, Ill., gave no spectrographic evidence of yttrium or the rare earths. This solution, which contained approximately 10 mg. of thorium per ml., was standardized by evaporating 10-ml. aliquots to dryness and igniting (to ThO_2) at 1000° C. to a constant weight. Dilutions were made of this solution to obtain a solution containing 10 micrograms of thorium per ml.

Apparatus and Technique. A Beckman Model DU quartz spectrophotometer with 1-cm. path length cells was used for all measurements. All solutions were diluted to a final volume of 10 ml. before being transferred to the absorption cells. The spectral band width ranged from 1.6 to 2.0 mμ for the spectral absorption curves, Figure 1. A spectral band width of 2.0 mμ was used to obtain a standard calibration curve.

Absorption Curves. The measurement of the spectral absorption of a water solution of the organic reagent (*A*, Figure 1) and of a similar solution containing thorium (*B*, Figure 1) indicated a shift of the absorption band toward longer wave lengths upon the addition of thorium. Water was used as the reference for these measurements. When the solution of reagent plus 67 micrograms of thorium was measured against the solution of reagent (*C*, Figure 1), the predicted curve indicating a maximum absorption at 545 millimicrons was obtained. The solutions containing 1 ml. of a 0.1% aqueous solution of the reagent were adjusted to a pH of approximately 0.5 with hydrochloric acid in a 10-ml. final volume. The data are plotted in Figure 1.

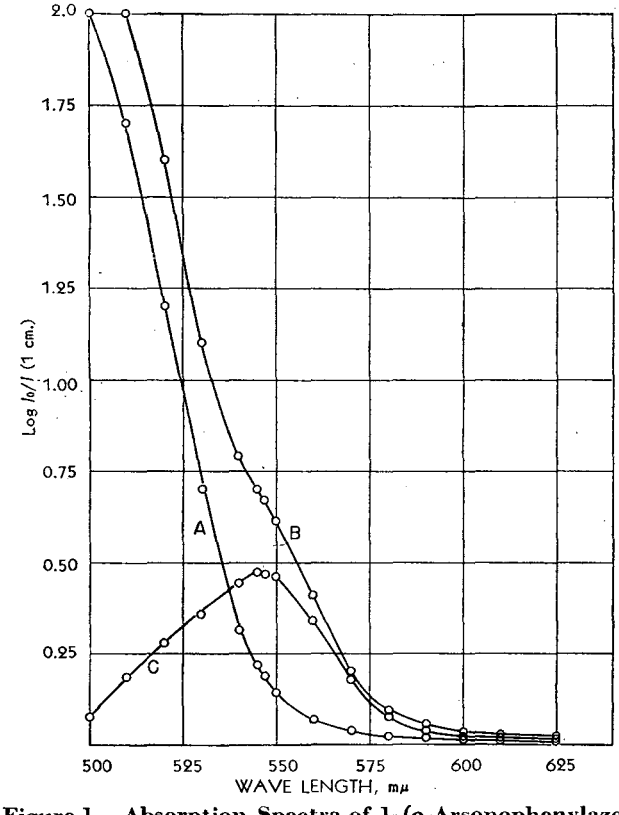

Figure 1. Absorption Spectra of 1-(*o*-Arsonophenylazo)-2-naphthol-3,6-disulfonic Acid

A. Reagent against water
B. Reagent plus thorium against water
C. Reagent plus thorium against reagent

Effects of Various Amounts of Reagent. The absorption of a series of solutions of pH 0.5, each containing 67 micrograms of thorium but with varying amounts of the organic reagent, was measured to determine a suitable concentration of the reagent. The data plotted in Figure 2 indicate that 1.0 ml. of a solution 0.1% in organic reagent is sufficient to complex at least 70 micrograms of thorium. Experience with later batches of the reagent indicated differences in purity and moisture content. Therefore, it is recommended that absorption curves be obtained for each batch of reagent to determine the amount required by 80 micrograms of thorium.

Effect of pH. A series of solutions containing thorium and the organic reagent was adjusted to various pH's with hydrochloric acid. Each of these was measured against a pH 0.5 reference solution containing only the reagent. A series of reference solutions containing only the reagent at various pH's was measured against distilled water. Between a pH of 0.2 and 1.0, the absorption remained essentially constant, as indicated in Table I. However, above pH 1.0 the absorption decreased rapidly and approached zero at pH 7.

The data show that in the pH range of 0.3 to 1.0 the maximum error due to variation in pH is about 2%.

Stability of Color. Solutions of thorium and organic reagent show no intensity variation over periods of time up to 24 hours. The colored solution obeys Beer's law, giving a standard curve that is a straight line through the origin with an optical density of 0.53 for 80 micrograms of thorium. When the amount of thorium exceeds 300 micrograms in a 10-ml. volume, a slight turbidity is detected. One milligram, or over, of thorium in a 10-ml. volume will form a red gelatinous precipitate which settles rapidly upon standing.

Interfering Substances. The interference from the UO_2^{++} ion increases with increasing pH as shown in Table II.

The absorption of various cations with the reagent was measured against a reagent reference (Table III).

Table I. Variation of Optical Density with pH

1 Ml. of 0.1% Organic Reagent in Water plus 43 Micrograms of Thorium, Measured *vs.* Reagent		1 Ml. of 0.1% Organic Reagent Diluted to 10 Ml. with Distilled Water, Measured *vs.* Water	
pH	Optical density	pH	Optical density
0.20	0.295	0.22	0.220
0.28	0.300	0.30	0.218
0.30	0.303	0.42	0.218
0.49	0.300	0.52	0.220
0.56	0.307	0.65	0.216
0.76	0.310	0.85	0.220
1.05	0.300	1.03	0.218

Table II. Interference of Uranium

(5000 micrograms as UO_2^{++})

pH	Optical Density	Thorium Equivalent, γ
0.40	0.068	9.5
0.50	0.078	12.0
0.92	0.185	26
1.10	0.342	49
1.45	0.76	>85
1.68	0.94	>85
1.75	1.12	>85

Table III. Absorption of Various Cations

Cation	Amount in 10-Ml. Vol., γ	Optical Density	Thorium Equivalent, γ
U^{++++}	20	0.050[a]	7
U^{++++}	40	0.120[a]	16
U^{++++}	80	0.275[a]	40
Zr	22	0.050	7
Zr	44	0.108	15
Zr	88	0.195	33
Fe^{+++}	500	0.058	7
Fe^{+++}	1000	0.107	15
Fe^{+++}	2000	0.137	20
La	5000	0.125	8
La	10,000	0.252	17
Yttrium	1000	0.102	15
Yttrium	3000	0.305	45
Yttrium	5000	0.520	72
Ti	1000	0.020	2.5
Ti	3000	0.050	9.0
Ti	5000	0.095	14.0
Ce^{+++}	1000	0.012	2.0
Ce^{+++}	3000	0.040	6.0
Ce^{+++}	5000	0.080	12.0
Ce^{++++}	1000 (Bleaches reagent completely)		

[a] Uranium (IV) complex with reagent seems unstable, as same solutions measured after 2 hours gave optical density of 0.022, 0.080, and 0.195, respectively.

Anions that complex thorium inhibit the formation of the colored complex. Fluorides, oxalates, phosphates, and large amounts of sulfates must be absent or removed.

Analytical Procedure. The following procedure is followed in this laboratory for the analyses of samples which may contain organic material or tetravalent uranium.

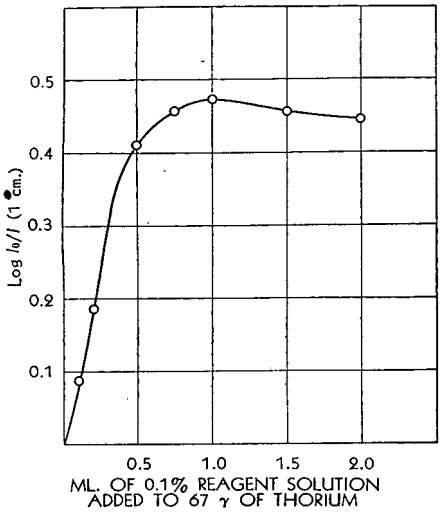

Figure 2. Effect of Various Amounts of Reagent

Each measured against reference containing an equal amount of reagent

Pipet a volume containing 5 to 80 micrograms of thorium into a small beaker. Take to dryness on a hot plate and make one or more evaporations with nitric acid. Add 0.5 ml. of concentrated nitric acid, 0.5 ml. of water, and 1.0 ml. of 70% perchloric acid and evaporate to dryness. Take up in 5 drops of concentrated hydrochloric acid and transfer to a 10-ml. glass-stoppered, volumetric flask.

Add 1.0 ml. of a 0.1% aqueous solution to the organic reagent. Make up to 10-ml. volume and measure at 545 mμ in a spectrophotometer against a reference solution. The reference solution is made by adding 5 drops of concentrated hydrochloric acid and 1.0 ml. of the organic reagent to a 10-ml. volumetric flask and making up to volume with water.

Elimination of Interferences. Interference from tetravalent uranium and organic compounds that complex thorium are minimized or eliminated by the nitric-perchloric acid step of the procedure. Relatively large amounts of fluoride may be eliminated by carrying the thorium with a hydroxide precipitation of a few milligrams of aluminum.

Synthetic samples containing varying amounts of thorium with 8 mg. of sodium fluoride and 4 mg. of aluminum were analyzed by adding ammonium hydroxide to precipitate the aluminum and thorium, centrifuging, and dissolving the precipitate in dilute hydrochloric acid. The precipitation was repeated three times. The organic reagent was added to the dissolved precipitate, the pH adjusted to 0.5, and the absorption determined in the usual manner. The results are shown in Table IV.

Ferric iron interference can be minimized by reduction to the ferrous state. This can be done by boiling the sample for a few minutes with 1 ml. of 10% hydroxylamine hydrochloride. According to the data shown in Table V, 50 micrograms of thorium can be determined in the presence of 1000 micrograms each of uranium and iron with an error of less than 5% by this procedure.

DISCUSSION

In the course of analyzing several hundred samples for thorium, a number of synthetic samples of known composition have been analyzed simultaneously with the actual samples to test the reliability of the thorium determination in the presence of other elements. The results of these analyses are shown in Table VI.

This reagent, while not as specific as might be desired, has been very useful for routine analysis of solutions containing relatively large amounts of aluminum and calcium.

Table IV. Estimation of Thorium after Separation from Sodium Fluoride

Sample No.	Thorium Present, γ	Thorium Found, γ	Recovery, %
1	10	9.5	95
2	30	28	93
3	50	48	96
4	70	67	96

Table V. Determination of Thorium in Presence of Uranium and Iron

Sample No.	Sample Composition, γ	Thorium Found, γ
1	Th 50 Fe 1000	50
2	Th 50 Fe 2000	50
3	Th 50 U 500 Fe 1000	50
4	Th 50 U 1000 Fe 1000	51

SUMMARY

A rapid colorimetric method for the determination of thorium in microgram quantities has been developed. Thorium can be determined in the presence of 20 times its amount of uranium and iron with an error of less than 5%.

Table VI. Analysis of Synthetic Samples

Sample No.	Sample Composition: Thorium, γ		Thorium Found, γ	Recovery, %
1	55.5	10 mg. NH_4Cl	54.0	97
2	49.2	5 mg. NaCl	49.0	99
3	12.3	1 mg. U + 10 ml. Cellosolve	13.8	112
4	49.2	1 mg. U + 1 mg. Fe^{+++}	51.0	104
5	49.2	1 mg. Zn	49.8	101
6	37.0	16 mg. Ca	36.0	97
7	49.2	1 mg. Cu	49.2	100
8	49.2	1 mg. Ni	49.0	99
9	49.2	1 mg. Pb	50.0	101

ACKNOWLEDGMENT

The authors wish to thank B. H. Ketelle for his translation of the Russian article, D. M. Black for his aid in preparing the reagent, and M. T. Kelley for his helpful suggestions.

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Humidity Testing Cabinet and Improved Humidity Regulator

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Close humidity regulation was achieved by letting steam into a cabinet through a solenoid valve controlled by a humidity-sensitive resistor. A description and wiring diagram for a sensitive relay activated by resistance variations are given.

IN TESTING the keeping ability of packaged food products, it has become customary to store the packages in a humidity cabinet kept at 100° F. and 90% relative humidity (*1*, *2*). These values of temperature and humidity are apparently used because they represent the worst conditions under which food products are kept in the temperate zone. Recently it was found necessary to enlarge the testing facilities in this laboratory and information about various commercially available testing cabinets was examined. As the previous testing cabinet had not controlled satisfactorily, and the commercially available cabinets not only were expensive (about $2000 apiece) but probably would not afford adequate control at 90% relative humidity, it was decided to build a thermohumidistat.

The case from a second-hand 20-cubic foot refrigerator was used as a cabinet, and an air-circulating system consisting of a blower and the appropriate duct work was constructed. The temperature was controlled by means of a de Khotinsky thermoregulator (placed in the cabinet), to which was connected a relay suitable for carrying the current to the heating elements. The amount of moisture in the air was adjusted by adding steam, which was introduced through a solenoid valve and orifice from a low pressure steam line. The orifice was chosen so that, when the system was in operation, the solenoid valve would be open for about one third of the time.

Control of the humidity was effected by the use of a moisture-sensitive resistor and an electrical system in which a variation in the resistance of the resistor caused a change in the deflection of a microammeter. The microammeter then acted as a switch to activate the solenoid valve on the steam line.

When the apparatus was first constructed, platinum contacts were attached to the indicating needle of a standard 0-100 microammeter to form a switch, and this switch controlled the grid bias of a vacuum tube relay. However, after the system had been in operation for a while, trouble was caused by sticking of the platinum contacts. A Symplytrol meter relay with a full scale deflection of 100 microamperes, manufactured by the Assembly Products Corp., Chagrin Falls, Ohio, was then used in the system, as shown in Figure 1. This meter relay is a very interesting piece of apparatus in which a contact is made between the indicating needle of the meter and an adjustable pointer that can be set to any point on the meter scale by a control knob. Once the contact is made, an electromagnet locks the contacts together until the current in the locking circuit is interrupted. When the locking current is interrupted, a spring snaps the contacts apart so that sticking cannot occur. A circuit for automatically interrupting the locking current once every 2 seconds is shown in Figure 1. With a 200-mfd. condenser across the contacts of relay B_1, the steam valve did not open with every interruption of the locking circuit. Although it is possible to construct humidity-sensitive resistors from thin films of hydrophilic polymers containing a small amount of inorganic salt, an uncalibrated Aminco-Dunmore humidity sensing element (American Instrument Co., Silver Springs, Md.) (gray color code) was employed, as it is very sensitive in the proper humidity range.

In adjusting the humidity controller, resistance R_1, Figure 1, was set to give a full scale reading when the humidity-sensitive element was shorted by switch *S*. Then the position on the microammeter at which contact was made was adjusted so that the humidity, as measured by wet and dry bulb thermometers in the cabinet, remained at the correct value.

In order to achieve good control, it is necessary to have an input of heat and steam sufficiently large to overcome quickly the effects of opening the cabinet doors. This means that the system may be flooded with heat or steam in case something goes wrong, and, therefore, it is advisable to use a safety device. Because both excess heat and excess steam raise the temperature in the cabinet, a thermorelay was arranged to activate the solenoid of a latching relay if the temperature were to rise about 105° F. This relay would then turn off the supply of electricity to the entire cabinet and activate an alarm. No provision was made to take care of the type of breakdown in which the heat or steam goes off, as this type of trouble is equivalent to removing the samples from the testing chamber and results only in lost time without destruction of the samples.

The entire cost of building this equipment, including labor, was

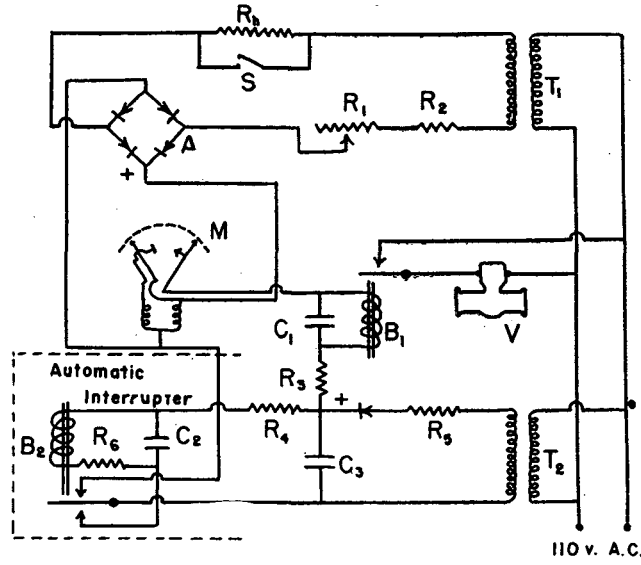

Figure 1. Wiring Diagram for Humidity Controller

B_1.	Plate current relay (5000 ohms)
B_2.	Plate current relay (7000 ohms)
C_1.	200-mfd. electrolytic condenser
C_2.	100-mfd. electrolytic condenser
C_3.	100-mfd. electrolytic condenser
M.	Symplytrol contact meter
R_h.	Humidity-sensitive resistor
R_1.	800,000 ohms
R_2.	500,000 ohms
R_3.	6800 ohms
R_4.	460 ohms
R_5.	47 ohms
R_6.	3300 ohms
T_1.	Constant voltage transformer (1:1 ratio)
T_2.	Isolating transformer (1:1 ratio)
V.	Solenoid valve for steam (normally closed)

[1] Present address, Great Lakes Carbon Corp., Morton Grove, Ill.

less than $500, and it has now been operating satisfactorily for several months. In operation the temperature and humidity controls go on or off approximately once a minute, and the wet and dry bulb temperatures do not vary more than 0.2° F. during the cycle. Although the cabinet was designed to operate at 100° F. and 90% relative humidity, it can be used at other values of temperature and humidity by readjusting the controls and using the appropriate humidity-sensitive resistor.

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RECEIVED January 29, 1949.

Apparatus for Measurement of the Vapor Pressure Lowering of Solutions

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A new apparatus is presented for measuring the vapor pressure lowering of solutions. The principle on which the apparatus operates is the vaporization under tension of the solvent from a solution at the boiling point of the pure solvent. The tension is applied by means of a liquid head.

THE apparatus described below was used to compare, at the same temperature, the pressures of boiling of a pure solvent and of a solution of nonvolatile solute in the solvent. In this way the vapor pressure lowering of the solvent by the nonvolatile solute was obtained. The apparatus was tried on two solvents and two solutes and found to be satisfactory.

EXPERIMENTAL

The apparatus as finally constructed is shown in Figure 1.

The solution chamber, *A*, was made by sealing off the female end of a 45/50 standard-taper joint so that the over-all length was 15 cm. The male end of the joint was sealed so that its total length was about 8 cm. At one side of the top of the male joint was sealed a 10/30 female standard-taper joint, *B*, for the insertion of a thermometer fitted with the corresponding male fitting. A 6-mm. stopcock, *C*, sealed into the top of the male joint, made connection with a Hyvac pump through a vapor trap fitted with ground-glass joints. A 1-mm. capillary bore stopcock, *D*, was sealed through the male joint so that one end extended to the bottom of the solution chamber. The other end was fitted with a 12/1 ground-glass spherical joint for connecting with the vapor chamber. These three seals were symmetrically arranged. The solution chamber was heated with a rheostat-controlled, detachable heating jacket which extended nearly to the bottom of the ground-glass joint. The thermometer had a range of −10° to 250° C.

The vapor chamber, *V*, was not a chamber in the ordinary sense of the word, but was that end of the tube in which vaporization took place and which was used in measuring the vapor pressure lowering. This tube, *E*, was made of 1-mm. bore capillary tubing bent in the shape of an inverted U. One end of the U, 45 cm. long, was used for measuring the liquid head from which the vapor pressure lowering is calculated. The tube was graduated in millimeters below the level of the vapor chamber stopper and ended in a stopcock, *H*, which made it possible to close the tube quickly and thus maintain any desired liquid head in the tube. The other end of the U was curved upward to form the vapor chamber, and was closed by a ground-glass mercury-sealed stopper held firmly in place by springs and fitted with a small glass ring at the top. The inverted U was connected at its apex to the solution chamber through a tube bearing the 12/1 female ground-glass spherical joint. The details of the vapor chamber are shown in the insert in Figure 1.

F was a Menzies boiling jacket, to which a small condenser was sealed as shown in Figure 1. The jacket was heated with an electric cone heater.

The vapor trap, *G*, was 12 cm. long and 2 cm. in diameter, fitted with a ground-glass stopper and 10/30 female standard-taper joint for connecting with stopcock *C* of the solution chamber. The trap was filled with glass rings to provide greater condensation surface.

Merck's thiophene-free benzene was purified according to the procedure of Washburn and Read (*3*). The middle fraction boiling at 78.50° C. and 720.0-mm. pressure was used.

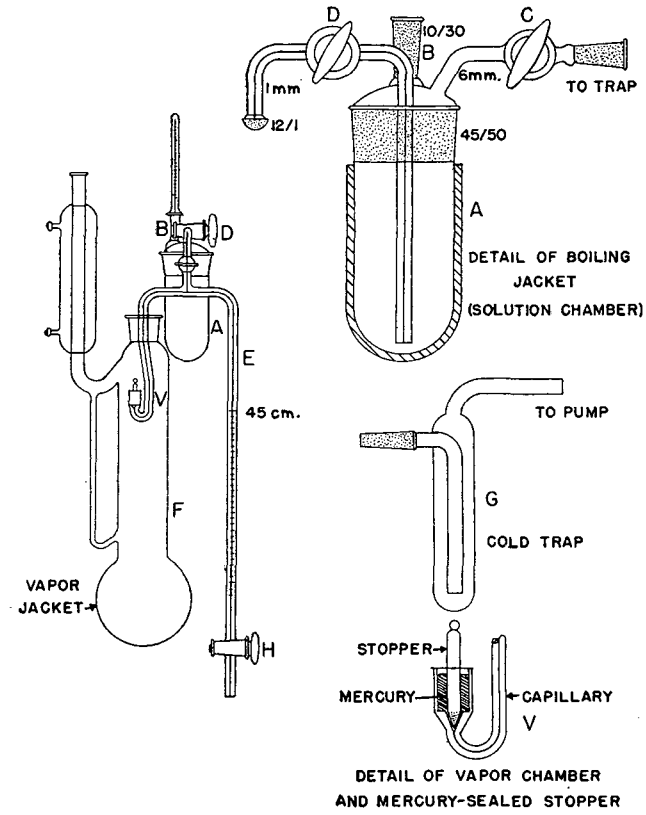

Figure 1. Diagram of Apparatus

Merck's C.P. carbon tetrachloride was purified according to the instructions of Cameron (*1*) followed by distillation over calcium oxide in an all-glass apparatus. The middle fraction boiling at 74.53° C. and 727.0-mm. pressure was used.

Anthracene was Eastman Kodak practical grade, recrystallized three times from 95% ethanol and dried several days in a vacuum desiccator. The product consisted of cream colored crystals melting at 217.0–217.5° C.

Crude benzil from a student preparation was recrystallized four times from 95% ethanol by saturating a boiling alcohol solution and adding distilled water. The product, dried several days over

calcium chloride in a vacuum desiccator, consisted of pale lemon-yellow needles melting at 95.0–95.2° C.

PROCEDURE

In operation, the vapor trap is carefully cleaned, dried in an oven, and weighed before being connected into the system. It is cooled in a Dewar tube containing ice slush. A solution containing the solute whose molecular weight is desired is prepared, using a weighed amount of solute and a measured volume of solvent. The solution is placed in the solution chamber, the ground-glass top with thermometer in place is inserted, and connection is made with the vapor trap. Stopcocks C and D are closed. The vacuum pump is started and C is opened slightly until the solution begins to boil at the reduced pressure. During this procedure, the bottom of D acts as a boiling tube and prevents bumping. C is then closed and a pinchclamp is placed between the vapor trap and the pump to prevent the pump from acting on the condensed solvent vapors in the trap. The heating mantle is slipped over the solution chamber and the solution is heated to slightly above the boiling point of the pure solvent as shown on the thermometer. The pinchclamp and stopcock C in the vacuum pump line are opened and the solution is degassed. This procedure is repeated at least twice at the elevated temperatures.

The trap is disconnected and weighed to determine the loss of solvent from the solution. This is necessary in order to determine the correct concentration of the solution used. In some instances, the solution chamber as a whole was weighed before and after the degassing procedure and in this manner correction was made for the solvent lost during degassing. Connection was then made to the inverted U by means of the spherical joint, which was tightly clamped. During the degassing process, the stopper to the vapor chamber was properly seated and sealed with mercury in order to prevent solvent condensing around the seal from seeping into the vapor chamber when the contained solution was under tension. This is the case when the liquid head in the long side of the U is below the liquid surface in the vapor chamber.

The stopcock on the long side of the inverted U is closed and the U is connected to the solution chamber by opening stopcock D. The solution, heated by the rheostat-controlled heater until its vapor pressure is about 10 mm. above atmospheric pressure, is forced by its own vapor pressure into the vapor chamber side of the U by slightly lifting the mercury-sealed stopper by means of the ring. This procedure removes any gases adhering to the walls of the tubing and vapor chamber. Owing to the pressure on the solution, there is no tendency for mercury to enter the vapor chamber when the stopper is loosened. Stopcock H is slightly opened and solution is allowed to enter the long arm to a point opposite the liquid surface in the vapor chamber. Stopcock D is closed and the vapor chamber is inserted into the boiling jacket. A split cork stopper, the halves of which are wrapped in tinfoil to prevent extraction of materials from the stopper by the solvent vapors, is used instead of a glass joint to close the top of the boiling jacket. This stopper makes the apparatus less rigid and less apt to be broken during manipulation. Many times it is desirable to flush the solution from the vapor chamber for duplicate determinations; by using a small hook in the ring of the mercury-sealed stopper, this can be done without removing the vapor chamber from the boiling jacket. The boiling jacket, containing pure solvent, is heated with the cone heater until the solvent boils and is refluxing steadily in the condenser. The cold reflux returns to the boiling jacket through the side tube and therefore never comes in contact with the vapor chamber. The vapor chamber and contained solution are thus maintained at the temperature of condensation of the pure solvent vapors.

With the liquid levels in the two arms of the U at the same point, the long arm of the U is tapped sharply with a pencil. This procedure tests the effectiveness of degassing, for any permanent gases retained by the solution will appear at this point in the vapor chamber, as bubbles which will coalesce and force the solution out of the vapor chamber. If no bubbles form, degassing may be assumed to be complete. With H wide open, D is slightly opened to give a slow, controlled rate of flow of the solution from the solution chamber into the long arm of the U. As the solution level in the long arm falls, the tube is continuously tapped until small bubbles of solvent vapor appear in the vapor chamber. D is at once closed and the bubble size is observed. If the bubble is not too large (a small bubble at the top of the vapor chamber, less in diameter than the bore of the tubing) and remains constant in size, the solution in the vapor chamber is at its boiling point under the reduced pressure in the chamber. The value of this reduced pressure is the difference between atmospheric pressure and the pressure equivalent of the tension exerted on the liquid in the vapor chamber by the column of liquid in the long arm of the U below the level of the vapor chamber. The pressure equivalent of the tension is used in calculations, as it represents the vapor pressure lowering of the solvent due to the solute dissolved in it. If the vapor bubble is observed to collapse, the pressure on the solution is still too great to allow the solution to vaporize at the boiling temperature of the pure solvent. In this case, stopcock D is carefully opened and the solution level in the long arm is lowered approximately 1 mm. D is closed and the tube is tapped to form another bubble. The observations described above are made on this bubble and the process is repeated until the bubble formed remains constant in size.

If the tension is such that the solution in the vapor chamber is above its boiling point, the bubble formed will enlarge and force the solution out of the chamber. If this happens, pressure is applied through H and simultaneously the mercury-sealed stopper is loosened to force solution through the vapor chamber. H is closed and the flushing procedure is repeated to bring fresh degassed solution into the vapor chamber. The boiling pressure is again determined as described above.

Duplicate determinations on the same solution are made until satisfactory reproducibility is obtained. It is hardly feasible to use all of the solution in the solution chamber for making duplicate determinations unless careful correction is made for the increasing amount of solvent existing as vapor in the solution chamber and for the consequent change in concentration of the solution.

THEORY

A solution boils at the same temperature as the pure solvent if the pressure on the solution is lowered sufficiently. The pressure lowering necessary may be considered directly proportional to the molality of the solution over a small range of temperatures near the boiling point.

The Clausius-Clapeyron equation for finite changes can be used for the calculations with data obtained from this apparatus.

$$\frac{\Delta T}{\Delta p} = \frac{RT^2}{LP} \tag{1}$$

This equation may be interpreted as giving the rate of change of boiling point with pressure at any total pressure P where L is the molal heat of vaporization at that pressure. $\Delta T/\Delta p$ may be evaluated for any given solvent at its boiling point. Assuming that the density of the solution is the same as that of the solvent, which for dilute solutions is a good approximation, the evaluation at atmospheric pressure is

$$\frac{\Delta T}{\Delta p} = \frac{1.987\ T^2}{760L} \times \frac{\mathrm{d}}{13.59} \tag{2}$$

where T is the boiling point of the pure solvent having density d at the temperature of the long arm of the U. For more exact work the density of the solution could be determined and used in the above calculations.

From ebullioscopy we have the following equation relating the boiling point elevation, ΔT, to the molality, m, of the solution

$$\Delta T = K_b m \tag{3}$$

where K is the molal boiling point constant. From the assumed linear relationship between temperature and vapor pressure of a substance near its boiling point, the following equation may be written:

$$T = kh + \text{constant} \tag{4}$$

where h is the pressure in millimeters of solvent and k is a constant factor of the order of $\Delta T/\Delta p$ of the solvent at its boiling point. For small changes

$$\Delta T = k\,\Delta h \tag{5}$$

where Δh is the liquid head necessary to cause vaporization of the solvent from the solution in the vapor chamber at the boiling point of the pure solvent, and ΔT is the corresponding elevation of boiling point.

Now by definition

$$m = \frac{w_2\ 1000}{w_1 M} \tag{6}$$

where w_2 is the grams of solute of molecular weight M dissolved in w_1 grams of solvent.

From Equations 3, 5, and 6

$$k\,\Delta h = K_b m = K_b \frac{w_2\ 1000}{w_1 M} \tag{7}$$

or

$$M = \frac{K_b}{k} \frac{1000\ w_2}{w_1\ \Delta h} = K' \frac{1000\ w_2}{w_1\ \Delta h} \tag{8}$$

and

$$K' = \frac{K_b}{k} = \frac{M w_1\ \Delta h}{1000 w_2} \tag{9}$$

Using weighed amounts of solute of known molecular weight in a weighed amount of solvent, K' can be determined from Equation 9. This known value of K' inserted in Equation 8 makes it possible to calculate the molecular weight of any other substance from vaporization data taken with this apparatus.

That Equation 8 is equivalent to the general ebullioscopic equation can be shown in the following manner. Substituting for k its equivalent $\Delta T/\Delta p$ from Equation 5 gives

$$M = \frac{K_b}{\Delta T/\Delta p} \frac{1000\ w_2}{w_1 \Delta h} = \frac{1000\ K_b\ w_2}{w_1\ \Delta T} \tag{10}$$

Table I. Vaporization Data for Solvent Benzene Using Solutes Anthracene (M.W. 178.2) and Benzil (M.W. 210.2) and for Solvent Carbon Tetrachloride Using Solute Benzil

Run	Solvent	Solute	Molality of Solution	Δh, Mm. of Solution	K' from Eq. 9
1	C_6H_6	Anthracene	0.0527	48.5	920
2	C_6H_6	Anthracene	0.0662	75.0	960
3	C_6H_6	Anthracene	0.0689	76.5	950
4	C_6H_6	Benzil	0.0241	23.0	945
5	C_6H_6	Benzil	0.0242	23.5	947
6	C_6H_6	Benzil	0.0485	45.5	935
7	C_6H_6	Benzil	0.0488	45.0	920
8	C_6H_6	Benzil	0.0963	88.5	913
9	C_6H_6	Benzil	0.0966	89.5	926
10	CCl_4	Benzil	0.0134	42.0	3100
11	CCl_4	Benzil	0.0266	82.0	3090
12	CCl_4	Benzil	0.0399	124.0	3110
13	CCl_4	Benzil	0.0433	132.0	3055

DATA

In Table I are recorded data obtained using the procedure described above.

Runs 1 to 3 show large deviations in K' because of an inadequate vapor trap. Runs 4 to 12 were taken with the improved trap described above, and give more consistent data. The average deviation of K' from the average in the first three runs is ± 16; for the next six runs is ± 12, and for all nine runs is ± 14. This is not the limit of accuracy obtainable with this apparatus, as is shown by the more precise data taken with carbon tetrachloride after the details of the technique had been more thoroughly worked out. Time precluded further experimentation with benzene after perfection of the technique. Much of the error in the first three runs resulted from an inadequate trap. The solution was analyzed after degassing and the concentration thus obtained was compared with the concentration of the solution calculated from the amount of solvent condensed in the trap. The data taken with the inadequate trap in these three runs showed the concentration as calculated from trap data to be about 2 to 3% below the actual concentration. The K' calculated would be correspondingly high. The analyses were made by drawing off solution into a cold vessel, weighing a portion, slowly evaporating the solvent in an oven, and weighing the residue of solute. Similar analyses and comparisons using the improved trap (Table II) show very close agreement between observed and calculated concentrations.

Table II. Concentration Data Obtained by Analysis and by Calculation from Trap Data Using Improved Trap

Grams of Solute per 1000 Grams of Solvent		% Deviation
Analysis	Trap data	Based on Analysis
20.56	20.57	0.05
20.48	20.50	0.09
20.22	20.22	0.00
15.88	15.50	0.14
10.25	10.23	0.10
5.52	5.51	0.20

Runs 10 to 12 using carbon tetrachloride as a solvent and benzil as solute show an average deviation from the average of ± 7. Run 13 was taken by weighing the entire solution chamber, using a triple beam balance in order to determine the weight of solvent lost in degassing. The rated sensitivity of this balance is 0.01 gram, but it is doubtful if its actual sensitivity exceeded 0.10 gram under the loading used. This method of weighing the solution chamber would perhaps be as accurate as weighing the condensation trap, if an analytical balance of sufficient capacity were obtainable. No attempt was made to push the method to the limit of accuracy, but a few factors would probably improve the data.

Correction could be made for the amount of solvent in the vapor state in the solution chamber. The volume of vapor could be found if the total volume of the solution chamber and the volume of solution at any time were known. The latter quantity could be determined if the solution chamber were constructed in the form of a narrow, graduated cylinder. The internal pressure could be approximated from the temperature to which the chamber is heated, as the vapor pressure of the solution would not differ markedly from the vapor pressure of the pure solvent at that temperature. A further advantage of calibrating the solution chamber would be that the correction for solvent vaporized during degassing could be made without the use of a trap or weighing the solution chamber. By noting the volumes before degassing and after filling the inverted U, determinations at different concentrations could be made with one filling of the solution chamber, for the concentration could be changed by vaporizing more solvent from the solution.

Because the inverted U tube is a capillary, the surface tension effects on the required liquid head should be accounted for. The effect of surface tension in the vapor chamber is to prevent the vapor bubble formed from pushing the solution down in the chamber and hence to collapse the bubble. The effect of surface tension in the long arm of the inverted U is to pull the liquid down in the tube and hence require less liquid head for vaporization than would be otherwise required. Because the vapor chamber is at the temperature of the boiling solvent and the long arm is at room temperature, these opposing effects will differ in magnitude. It is this difference in tension effects that should be accounted for.

Under ordinary conditions and without extreme precautions in making corrections, the apparatus is capable of giving data that compare favorably with those obtainable by standard commercial forms of apparatus used in making similar measurements. The Menzies apparatus (*2*) is used in making identical measurements. It is simpler in design and somewhat more rapid in manipulation, but lacks the certainty of complete degassing which is obvious in the authors' apparatus. Neither apparatus can attain any great degree of accuracy using solutes with appreciable vapor pressure at the boiling point of the solvent. For this reason benzil, boiling at 3° C., was used to evaluate K'.

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Determination of Carbon and Hydrogen by Combustion

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A simplified rapid method for the routine semimicrodetermination of carbon and hydrogen is presented. Liquid samples are slowly vaporized, and the vapors are burned in a flame at the tip of the containing ampoule. Combustion is supported by a stream of oxygen at a very high flow rate. A technique for handling solid samples is also presented, by which two sets of apparatus can be operated simultaneously, thereby allowing six to eight determinations per day; explosion hazards are diminished; and a shorter training period for new analysts is required.

IN RECENT years most of the progress made in the determination of carbon and hydrogen has been in the field of microchemistry. Desirable features of the apparatus developed by microchemists have often been adapted advantageously by analysts to routine samples of semimicro or macro size.

Tunnicliff, Peters, Lykken, and Tuemmler (*16*) reported that the merits of their routine macromethod employing the new dual-unitized apparatus "are principally due to the adaptation of previously known practices and to the emphasis placed on adequate control of the combustion operations, rather than to the development of new ideas and techniques." Speed was obtained mainly by the simultaneous combustion of two samples. Hallett (*6*), Royer, Norton, and Sundberg (*14*), and Steyermark (*15*) have reported successful use of motors to drive movable burners slowly and automatically over samples.

The procedure described herein presents a technique for burning liquid samples whereby the bulk of the sample is kept relatively cool and slowly vaporized by heat applied near the exit end of the sample ampoule. By supporting the exit end at a higher level than the body of the ampoule, it is possible to burn the vapors in a small flame at the tip without the danger of liquids entering the combustion chamber. A fast oxygen rate (300 to 350 ml. per minute) results in increased pressure which, according to Brodie (*3*), aids in producing completeness of combustion as well as lessening combustion and sweeping times. No trouble is experienced with condensation of the water in the tip of the combustion tube or in the entrance arm of the water absorber, as has frequently been reported by others. The sealed-in capillary feature used by Baxter and Hale (*1*) and adopted by others (*13*) was designed to accomplish this same purpose.

APPARATUS AND PROCEDURE

The apparatus employed in this procedure is illustrated in Figure 1. It consists of conventional type equipment arranged to conform to the technique described in this paper.

Preheater and Purifier. One preheater, which serves both sets of furnaces, consists of a KA-2 pipe 500 mm. long by 25 mm. in inside diameter, filled with copper oxide wire. The purifying train consists of two sections of KA-2 pipe in series following the preheater and filled with Ascarite and Dehydrite, respectively. The purified oxygen is piped from the preheater and purifying train by a delivery pipe of sufficient length (80 mm. being the minimum length suggested by Clark and Stillson, *5*) to allow cooling of the oxygen. A needle valve is provided for each set of apparatus.

Combustion Tube and Filling. The combustion tube is made of quartz, has an inside diameter of 20 mm., is 680 mm. long, and is reduced at one end to a tip 22 mm. long, 4 mm. in inside diameter, and 7 mm. in outside diameter. The combustion tube filling is shown in Figure 2.

Combustion Tube Heaters. Three heaters are used, as shown in Figure 1. The sole purpose of the primary heater is to supply heat to burn completely any carbonaceous residue remaining in the ampoule after the bulk of the sample has burned. All three heaters are hinged and slide on rods attached to the base of the multiple unit set. A device, shown in Figure 3, made from sheet asbestos and laboratory tongs, is placed adjacent to the front surface of the middle heater during the cooling of the tube, in order to prevent the stream of air from cooling the heater below its critical temperature. A similar device remains constantly in place adjacent to the exit surface of the final heater.

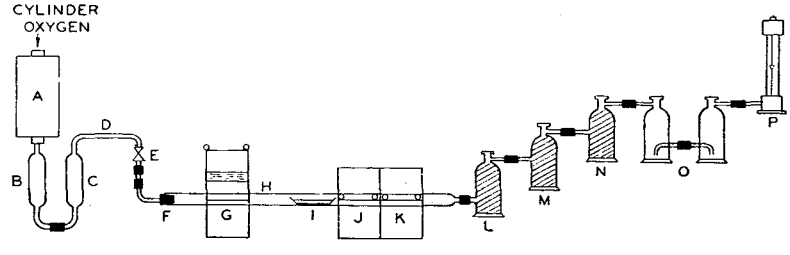

Figure 1. Apparatus for Determination of Carbon and Hydrogen by Combustion

A. Preheater
B. Ascarite absorber
C. Dehydrite absorber
D. Delivery line
E. Needle valve
F. Oxygen inlet
G. Primary heater (movable)
H. Quartz combustion tube
I. Combustion boat containing ampoule
J. Middle heater (movable)
K. Final heater
L. Dehydrite absorber
M. Ascarite absorber
N. Dehydrite-Ascarite absorber
O. Palladium chloride bubbler and trap
P. Rotameter

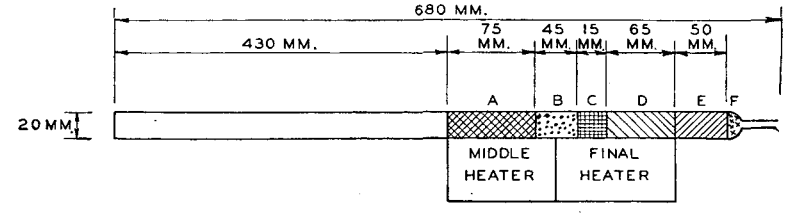

Figure 2. Combustion Tube Filling

A. Roll of copper gauze
B. Quartz chips
C. Platinized asbestos
D. Copper oxide wire
E. Load chromate on copper oxide
F. Glass wool

Absorption Train. The absorbers used are of the simple Midvale type, Stetser-Norton modification (7). The water absorber is filled with Dehydrite, and glass wool is employed in the bottom of the absorber and on top of the filling to avoid loss of finer particles. The carbon dioxide absorber is filled with Ascarite, except for a short section of Dehydrite which is placed on top of the Ascarite to prevent any possible loss of water vapor. Glass wool is placed above and below the filling in this absorber also.

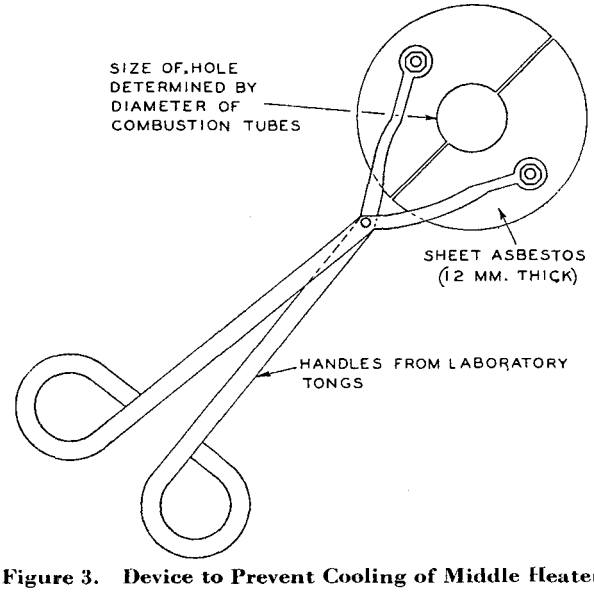

Figure 3. Device to Prevent Cooling of Middle Heater

In addition to the water and carbon dioxide absorbers, the absorption train includes an additional absorber filled with Dehydrite and a bubbler preceded by a trap. The bubbler contains a solution of palladium chloride (0.03 weight %) through which the exit gas passes. Thus, a continuous test for incomplete combustion is provided. A black precipitate indicates the presence of carbon monoxide. Because constant exposure to carbon monoxide in the air also discolors this solution, it should not be used for more than 2 to 3 days. A flowmeter is attached to the final bubbler preceded by an absorber containing Drierite and glass wool.

Procedure. LIQUID SAMPLES. Assemble the combustion units, fill and install the combustion tubes, fill and attach the absorbers, and adjust the heaters to operate at the following temperatures (±25° C.):

Primary heater	845° C.
Middle heater	900° C.
Final heater	760° C.
Preheater	650° C.

Purge the combustion train with oxygen at a rate of 300 to 350 ml. per minute until a 1-hour blank does not exceed 0.5 mg. of water or carbon dioxide. Do not alter the valve setting during an analysis. After a minimum of 15 minutes' flushing with oxygen remove the absorbers from the train, place a rubber policeman over each exit arm, and allow the absorbers to stand in an enclosed box near the balance. Remove the rubber policemen from the arms of the absorbers immediately prior to weighing. Lightly touch a chamois to the entire surface of the tare and to each absorber prior to its first weighing on a given day. Do not wipe absorbers at other times during the day except when necessary. Weigh the absorbers to ±0.1 mg. by direct comparison with a tare which has been prepared using a container similar to the absorbers. When not in use, the tare is allowed to stand in the balance case.

Take a sample of approximately 0.1 gram, weighed to ±0.1 mg., for the analysis. Weigh liquid samples in ampoules made from 40-mm. sections of 5-mm. outside diameter Pyrex tubing sealed at one end to a 30-mm. length of glass rod and drawn out to a capillary at the other end (see Figure 4). An inside diameter of the capillary for use in analyses of light samples (A.P.I. gravity 50+) may be as small as 0.7 mm.; for use in analysis of heavier samples (A.P.I. gravity 50−) it should be larger (approximately 1.0 mm.). The capillary walls of all ampoules should be of minimum thickness, 0.3 mm. After proper selection of the ampoule for use in analysis of a given sample, break the capillary at a point 50 mm. from the sample section. Wipe the ampoule with a chamois and weigh.

To fill the ampoule, warm it in a small flame and then cool it with dry ice while the capillary is immersed in the sample. Shake the ampoule so that all of the sample lies in the end opposite the capillary. A 15-mm. column of heavy material or a 25-mm. column of light material in the main part of the ampoule provides the desired weight of sample. Wipe the tube with a cleansing tissue to free it from condensed water vapor from the air and from traces of the sample. Hold the tube approximately 2.5 cm. (1 inch) from the side of a Bunsen burner flame with the bubble created between the wanted and unwanted portions of the sample nearest the heat. Hold a cleansing tissue at the end of the capillary to absorb the excess sample as it is driven out. Dry the entire capillary by passing it lightly through the flame, bend it back slightly at the end to form a hook for hanging it in the balance, and seal the tip. After permitting the ampoule and sample to reach the temperature of the room, weigh the sample. Do not permit the sample to enter the capillary tip of the ampoule at any time after filling.

Place the weighed absorbers, joined together (glass to glass) by means of rubber tubing approximately 1.9 cm. (0.75 inch) in length, in the train. Move the primary heater along the slide rod as far as possible from the middle heater. Place the asbestos device shown in Figure 3 adjacent to the front surface of the middle heater. Clamp a removable air jet to the front slide rod and direct a strong stream of air at a point on the tube 10 or 12.5 cm. (4 or 5 inches) distant from the asbestos device. Place cold damp cloths over the sample portion of the tube and the open primary heater until the sample portion is cool to the touch. Break the capillary of the ampoule at a point 25 mm. from the enlarged portion of glass containing the sample. Place the portion thus removed in an ignited boat and place the ampoule in an inclined position in the boat (see Figure 4) so that the capillary remains above the liquid level of the sample, its tip pointing toward the middle heater. Place the boat in the tube so that the tip of the ampoule is within 2.5 cm. (1 inch) of the rolled copper gauze. Remove the asbestos device and air jet. Place the damp cloth on the tube, over the sample.

Maintain a very small continuous-burning flame at the tip of the ampoule by gradually moving the middle heater toward the sample as the cloths are moved backward. Raise the lid of the middle heater slightly from time to time to observe the flame.

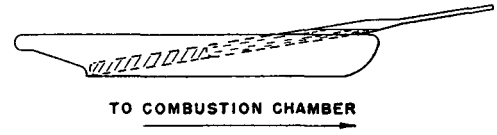

Figure 4. Combustion Boat Containing Sample Ampoule

When the bulk of the sample has burned, move the middle heater to a position adjacent to the stationary final heater; place the primary heater over the ampoule and return the middle heater to a position adjacent to the primary heater. Turn on the primary heater. (Often the capillary tip seals over during an analysis. Application of additional heat increases the sample vapor pressure and causes a new opening to be blown through the molten glass. The sample vapors continue burning at this new opening.)

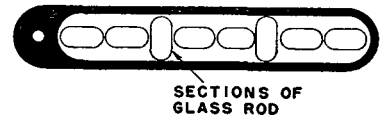

Figure 5. Compartment Boat for Burning Solid Samples

After 40 minutes, turn off the primary heater, remove the absorbers from the train, apply rubber policemen to the arms, and allow the absorbers to rest in the enclosed box for 20 to 40 minutes before being reweighed.

SOLID SAMPLES. Burn solids, or extremely heavy liquids, in a specially prepared porcelain boat made into a number of compartments by fusing into it 1-cm. sections of glass rod at 2-cm. intervals (see Figure 5). Weigh the same size of sample as in the case of liquids. The rate of combustion of the sample can be controlled by watching the flame or by watching the rate of disappearance of the sample, depending upon the nature of the material. Other parts of the procedure are the same as described above.

ACCURACY AND PRECISION

The accuracy of the method has been determined by analyses of one Bureau of Standards sample (benzoic acid) and several other

Table I. Accuracy and Precision Data for Pure Compounds

Compound	Carbon, Wt. %	Hydrogen, Wt. %	Compound	Carbon, Wt. %	Hydrogen, Wt. %
Iso-octane	84.22	15.96	Toluene	91.46	8.87
	84.15	15.83		91.40	8.90
	84.20	16.09		91.28	8.83
	84.16	16.02			
	84.05	15.84	Benzoic acid	68.84	5.01
	84.08	15.93		68.76	4.99
	84.33	16.01		68.70	5.03
	84.30	16.00			
	84.22	16.06	Synthetic mixture[a]	82.02	11.65
				81.87	11.69
Cetane	84.85	15.16		81.94	11.72
	85.01	15.23			
	84.77	15.16			

	Iso-Octane		Cetane		Toluene		Benzoic Acid		Synthetic Mixture	
	C	H	C	H	C	H	C	H	C	H
Computed value	84.12	15.88	84.87	15.13	91.25	8.75	68.84	4.95	81.92	11.60
Average value	84.19	15.97	84.88	15.18	91.38	8.87	68.77	5.01	81.94	11.69
Accuracy[b]	+0.07	+0.09	+0.01	+0.05	+0.13	+0.12	−0.07	+0.06	+0.02	+0.09
Precision, *S.D.*[c]	0.09	0.09	0.10	0.03	0.08	0.03	0.06	0.02	0.06	0.03

[a] Acetic acid 4.94 wt. %; ethyl acetate 4.93 wt. %; *n*-butyraldehyde 3.76 wt. %; methyl isobutyl ketone 3.25 wt. %; isoamyl alcohol 3.87 wt. %; iso-octane 17.64 wt. %; *n*-heptane 17.45 wt. %; toluene 44.16 wt. %.
[b] Average minus computed value.
[c] Standard deviation is defined as $S.D. = \sqrt{\frac{\Sigma da^2}{n}}$.

Table II. Precision of Procedure for Carbon and Hydrogen by Combustion

(Miscellaneous samples)

Sample No.	Carbon, Wt. %	Dev. from Average	Hydrogen, Wt. %	Dev. from Average
1	77.36	0.05	13.35	0.03
	77.26		13.29	
2	83.23	0.04	14.05	0.05
	83.15		14.13	
3	74.62	0.07	12.74	0.14
	74.48		12.93	
4	77.84	0.03	13.65	0.00
	77.79		13.65	
5	77.27	0.06	13.67	0.00
	77.39		13.67	
6	77.55	0.13	13.70	0.09
	77.82		13.53	
7	81.11	0.03	14.01	0.03
	81.05		14.07	
8	83.73	0.07	12.84	0.04
	83.86		12.92	
9	75.53	0.15	13.40	0.06
	75.23		13.28	
10	82.84	0.11	13.91	0.01
	83.07		13.89	
11	83.02	0.07	14.03	0.01
	82.88		14.06	
12	82.89	0.14	13.99	0.09
	82.62		13.82	
13	78.01	0.11	13.64	0.01
	77.78		13.62	
14	82.02	0.13	12.80	0.01
	82.28		12.78	
15	79.73	0.11	13.54	0.01
	79.50		13.57	
Av.		0.09		0.04

pure compounds. The results of these tests, shown in Table I, indicate that the accuracy is of the order of 0.06% for carbon and 0.08% for hydrogen.

The precision of the procedure was determined from the data in Table I; these show an average standard deviation of 0.08% carbon and 0.04% hydrogen. Duplicate analyses of fifteen miscellaneous plant samples are shown in Table II to indicate the precision to be expected on unknown complex mixtures.

The precision and accuracy of this method compare favorably with those of the routine procedures of microchemists. Steyermark (*15*) shows an acceptable accuracy of ±0.3% deviation from theory with many checks within a few hundredths of 1%. Belcher and Spooner (*2*) claim results comparable in accuracy with those obtained with general standard methods of microanalysis—0.2% for carbon and 0.1% for hydrogen. Powers (*12*), in his statistical study of accuracy and precision of analytical microdeterminations of carbon and hydrogen, quotes Niederl as saying that ±0.2% is acceptable with no values to exceed ±0.3%, and found in his own study an existing precision obtained from 349 determinations by 23 experienced microanalysts of 2.9 parts per 1000 of carbon and 22 parts per 1000 of hydrogen.

DISCUSSION

Combustion Tube Filling. The purpose of each constituent of the filling is:

Filling Constituent	Purpose
Lead chromate	Sulfur removal
Quartz chips	Provide large amount of hot contact surface
Copper gauze roll	Hot radiating surface enables sample vapors to reach flash point sooner and allows easy control of their rate of burning
Platinized asbestos	Catalyst for combustion reaction
Copper oxide wire	Catalyst for combustion reaction

Combustion Tube Heaters. Hinged-type heaters are found advantageous, particularly in the case of the middle heater, because the operator is able to raise the upper half of the heater slightly from time to time in order to view the small flame.

Absorbents. Dehydrite and Ascarite were chosen for use in this procedure. They offer the advantages of being solids; as Niederl and Roth (*11*) reported, Dehydrite absorbs up to 30% of its weight and Ascarite absorbs 10 times as much carbon dioxide as does soda lime.

Oxygen rates, using previously reported techniques, were necessarily slow in order to allow sufficient residence time for complete combustion in the catalyst-packed combustion tube, inasmuch as the burning did not all take place at the capillary tip of the ampoule as is the case in the present method. Rates ranging from 50 to 250 ml. per minute (*2, 8*) were considered fast. Millin (*8*) found complete absorption of water by Dehydrite and of carbon dioxide by Ascarite at 250 ml. per minute. A test at these laboratories of the carbon dioxide absorption capacity of Ascarite showed complete absorption at rates up to 1000 ml. per minute, employing an absorber of the Midvale type, Stetser-Norton modification (*7*).

Extreme care in properly packing the Ascarite (8- to 20-mesh) is essential to prevent channeling. It is advisable to add the re-

Table III. Time Required for Two Simultaneous Analyses by Routine Procedure

Operation	Time, Minutes
Weigh absorbers (4)	8
Weigh samples (2)	8
Connect absorbers, insert samples	10
Burn all sample vapors	25 ± 10
Bring sample heater up to temp.	20
Burn carbon residue from ampoule	5
Flush combustion tube	15
Disconnect absorbers and all rubber policemen	5
Equilibrate absorbers	20
Weigh absorbers	8
Calculate results	3
Total elapsed time for two analyses	127
Total working time for two analyses	67

agent in small portions, tapping the absorber gently after each addition, as suggested by Clark and Stillson (*4*). Care in selecting, handling, and storing Ascarite is also essential, as some lots of this drying agent were not effective.

Time Required for Analysis. Table III gives the average time required for the various operations necessary for this procedure. Six to eight determinations can be made in an 8-hour day. The working time can be lessened somewhat by using weighed absorbers from one determination for a subsequent determination and using a smaller sample [50 to 70 mg. have been suggested by Natelson and co-workers (*9, 10*)].

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Chemical Determination of Tryptophan in Proteins

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Fundamental knowledge of the behavior of free and peptide-linked tryptophan is needed for the development of an accurate method of analysis of proteins for tryptophan. Described are: a method of alkaline hydrolysis which protects tryptophan from external destruction at temperatures up to 185° C. without addition of antioxidants to the solution; the effects of time and temperature on the racemization of free tryptophan heated in 5 *N* sodium hydroxide; and the effects of free and peptide-linked amino acids on tryptophan heated in 5 *N* sodium hydroxide. A variable proportion of tryptophan in proteins is destroyed by cystine, cysteine, lanthionine, serine, and threonine during alkaline hydrolysis but these amino acids do not destroy tryptophan under conditions used for analysis of unhydrolyzed proteins. Several modifications of a method for colorimetric analysis of unhydrolyzed proteins are described. The basic method, like that previously described for the determination of free tryptophan, involves two steps—reaction of tryptophan and *p*-dimethylaminobenzaldehyde in 19 *N* sulfuric acid to form a colorless condensation product and subsequent development of a blue color by oxidation with sodium nitrite. For greatest accuracy each protein must be analyzed using predetermined optimum conditions for the basic reactions. A general procedure, based on studies with eleven proteins, may be used where resulting economy of time outweighs the possible sacrifice of a small degree of accuracy. The precision of the method is ±0.88% and the accuracy is believed to be ±1 to 3%. Tryptophan may be determined in the presence of carbohydrates.

DIFFICULTIES in the accurate quantitative determination of tryptophan in proteins and the biological importance of this essential amino acid account for the contentious history of this subject. Whether proteins can be hydrolyzed with alkaline agents without significant destruction of tryptophan is still controversial. In this laboratory significantly higher values were obtained on the unhydrolyzed protein than on the alkaline hydrolyzate, even when hydrolysis was carried out by a procedure that eliminated external destruction of tryptophan. This observation led to a study of free and peptide-linked tryptophan under alkaline hydrolyzing conditions. The conclusion was that alkaline hydrolysis of proteins preliminary to tryptophan analysis should not be used because of variable amounts of destruction of tryptophan, depending on the amino acid composition of the protein.

Results of this study are recorded to explain the fundamental causes of destruction of tryptophan during alkaline hydrolysis of proteins and to provide evidence of the accuracy of the method described for the determination of tryptophan in proteins in which these destructive factors are eliminated. A critical evaluation of the accuracy of the values obtained by this method is also presented. Because of its biological importance, the scope and complexity of analytical problems concerning tryptophan are much broader than those involved in protein analysis. These results provide methods and fundamental information based upon which the analyst and the research worker may devise procedures to meet special conditions.

APPARATUS AND MATERIALS

A Coleman spectrophotometer, Model 11, was used for colorimetric analyses. A Beckman quartz spectrophotometer was used to obtain the absorption curves shown in Figure 6. Parr nickel microbombs are commercially available.

Reagents for colorimetric analysis have been described (*30*). Sodium hydroxide was prepared from a saturated "carbonate-

free" solution made from reagent grade sodium hydroxide. Hydrogen was freed from residual oxygen by passage through a 10-mm. Vycor glass tube which contained a section 200 mm. long filled with 10% platinized asbestos heated to about 700° C.

S. faecalis R. was obtained from American Type Culture Collection, Georgetown University School of Medicine, Washington, D. C.

Tryptophan. Analytically pure L-tryptophan, sample II (*30*), was used for standard curves. Another pure, recrystallized sample (III) of L-tryptophan which was microbiologically twice as potent as analytically pure DL-tryptophan was used for work involving microbiological analysis. The derivatives of L-tryptophan were analytically pure preparations made in this laboratory (*28*).

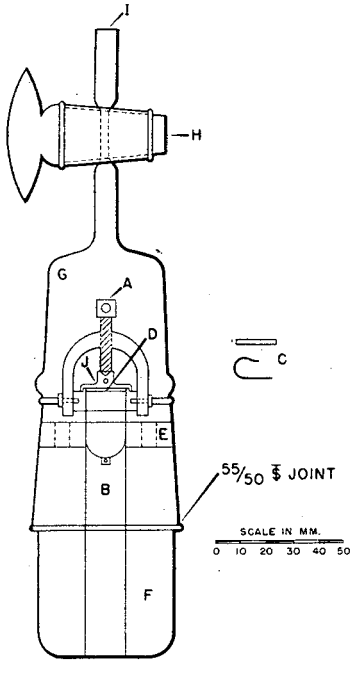


Figure 1. Hydrogen Filling Apparatus

Amino Acids and Carbohydrates. Commercially available amino acids of best quality were used. L-Cystine was purified by conversion to the hydrochloride and then precipitated from water and washed free of chloride ions. The nitrogen content was 11.55%; the theoretical nitrogen is 11.65%. Glucose was a National Bureau of Standards sample. Fructose was a purified sample.

Casein. Casein was prepared from fresh skimmed milk by the method of Van Slyke and Baker (*35*) as modified by Cohn and Hendry (*4*). All-glass apparatus was used and the pH was never higher than 6.3. The product was practically free from calcium and was free from chloride ions. The casein was dried in a vacuum over calcium chloride, ground in a Wiley mill to pass a 40-mesh sieve, and then equilibrated with air. The yield from 2 liters of milk was 45.3 grams. Analyses: ash, 0.79; water, 5.52; nitrogen, 15.72% (ash- and water-free basis).

β-Lactoglobulin. β-Lactoglobulin was prepared from defatted Jersey milk and crystallized according to the method of Sorensen and Sorensen (*27*) by W. G. Gordon. The sample was recrystallized in this laboratory as follows: The moist crystals were stirred up in 100 ml. of 0.12 molar sodium chloride solution and the slightly turbid solution was clarified by centrifugation and filtration through a hardened paper. The solution, pH 5.14, was dialyzed against distilled water until free from chloride ions. Crystallization occurred during dialysis. Microscopic examination showed uniform crystal structure similar to that shown in Figure 2 (*25*). The crystals were separated by centrifugation, washed once with 200 ml. of water, and dried in a vacuum over calcium chloride. The product was ground to pass a 60-mesh sieve and equilibrated with air. Analyses: ash, 0.09; water, 6.21; nitrogen 15.44% (ash- and water-free).

Native Crystalline Ovalbumin. A water solution of ovalbumin which had been recrystallized seven times by a modification of the method of Kekwick and Cannan (*14*), by E. J. Coulson, was used.

Heat-Denatured Ovalbumin. A solution of crystalline ovalbumin was dialyzed against distilled water until free from sulfate ions, clarified by centrifugation and filtration, and then heated on a steam bath at 100° C. for 1 hour. The suspension was centrifuged and the coagulated ovalbumin was washed once with 200 ml. of water. The product was dried in a vacuum over calcium chloride, ground to pass a 60-mesh sieve, and equilibrated with air. The yield was 2.46 grams. Analyses: ash, 0.02; water, 6.54; nitrogen, 15.50% (ash- and water-free).

Native Conalbumin. Conalbumin was prepared by the method of Longsworth *et al.* (*20*) and reprecipitated six times by E. J. Coulson.

Heat-Denatured Conalbumin. A solution of conalbumin was heated on a steam bath at 100° C. for 30 minutes. The suspension was cooled to 25° C. and the coagulum separated by centrifuging. The solid was washed twice with 100-ml. portions of water, dried in a vacuum over calcium chloride, and equilibrated with air after powdering. The yield was 343 mg. Analyses: ash, 0.29; water, 7.98; nitrogen, 16.47% (ash- and water-free). The preparations of conalbumin and ovalbumin were free from each other, as shown immunologically by the Schultz-Dale technique by E. J. Coulson.

Subtilin. The subtilin, obtained from J. C. Lewis, was ground to pass an 80-mesh sieve and equilibrated with air. Analysis: ash, 0.75%. Water was determined each day samples were weighed.

Other Proteins. Samples of edestin, arachin, ox muscle, and zein were obtained from D. B. Jones. Gelatin was a Difco product. CS-54R and CS-56R were allergenic polysaccharidic proteins from cottonseed (*29*) which contained 6.3 and 35.2% carbohydrate, respectively. The nitrogen contents of the proteins agreed well with literature values. Nitrogen was determined by a Kjeldahl micromethod using mercuric sulfate as catalyst. Water was determined by heating the sample for 3 hours in an Abderhalden vacuum dryer at 110° C. Ash was determined by ignition in an electric furnace for 1.5 hours, during which time the temperature rose to 750° C. All analytical results with proteins are expressed on an ash- and water-free basis.

A METHOD OF ALKALINE HYDROLYSIS FOR TRYPTOPHAN STUDIES

A procedure for alkaline hydrolysis at temperatures up to 185° C., which protects tryptophan from external destruction without addition of antioxidants to the solution, was developed for use with free tryptophan and tryptophan in proteins. A chemical micromethod was developed for analysis of hydrolyzates. The effect of time and temperature of hydrolysis on the value of the tryptophan content of casein was determined using both chemical and microbiological methods of analysis. The tryptophan content of several other proteins was also determined by chemical and microbiological analysis of their alkaline hydrolyzates for comparison with the values obtained on these same proteins by a method that starts with the intact protein.

CHOICE OF HYDROLYZING AGENT

In general, three principal alkaline hydrolyzing agents—sodium hydroxide, barium hydroxide, and sodium hydroxide–sodium stannite—have been used. Controversy over the relative merits of barium hydroxide and sodium hydroxide as protein hydrolyzing agents for tryptophan analysis has continued since the early work of Herzfeld (*12*), Homer (*13*), and Onslow (*24*). Barium hydroxide has the disadvantage that barium ions must be removed before analysis of the hydrolyzate. Sodium hydroxide–sodium stannite reagent, used by Lugg (*21*), is cumbersome because of the necessity for carrying out the hydrolysis in a sealed tube, removing tin and dissolved glass, and extracting the hydrolyzate with toluene. Brand and Kassell (*2*) showed that this reagent had no advantage over sodium hydroxide alone for the determination of tryptophan in ovalbumin and cattle fibrin. Cysteine was used by Kuiken *et al.* (*17*) to prevent oxidative destruction of free tryptophan on autoclaving in a sealed tube in sodium hydroxide.

Enzymatic hydrolysis of proteins has been used but critical demonstration that this procedure quantitatively liberates tryptophan from proteins without any destruction has not been made.

In this work, 5.0 *N* sodium hydroxide was used.

HYDROLYSIS PROCEDURE J

There is no general agreement on hydrolysis procedures for proteins. Glass vessels are universally used for refluxing or autoclaving in sealed tubes, but glass is appreciably soluble in hot alkali and must be removed before analysis of the hydrolyzate. Lugg (*21*) recommended evacuation of glass tubes before sealing, but Brand and Kassell (*2*) abandoned this procedure because of danger of loss of powdery proteins as the air was removed.

A satisfactory hydrolysis procedure requires (1) an inert vessel that can be used repeatedly over a wide range of temperatures; (2) protection of tryptophan from oxidation by an inert atmosphere, thus making unnecessary the addition of antioxidants that may interfere with reactions being studied or require removal to prevent interference with chemical or microbiological analysis of hydrolyzates; (3) prevention of sample loss on evacuation and filling with inert gas; (4) use of reproducible conditions; (5) preparation of sufficient hydrolyzate for both chemical and microbiological tests; and (6) use of 20 to 50 mg. of protein sample.

The procedure here described fulfilled these requirements. The Parr nickel microbomb (capacity about 3 ml.) was an ideal vessel for hydrolysis with sodium hydroxide at temperatures up to 185° C. Purified hydrogen was a suitable inert atmosphere. Insoluble proteins were compressed into pellets with a micropress, thereby eliminating possible loss on evacuation of the bomb.

This method of hydrolysis is applicable to protein, free tryptophan, and tryptophan plus added substances.

Charging the Bomb. For studies on free tryptophan 1 ml. of a solution of tryptophan in 5.0 *N* sodium hydroxide was placed in the nickel cup part of the bomb using a 1-ml. calibrated syringe. In dealing with proteins the sample, weighing 20 to 50 mg. and previously compressed into pellets of approximately 3 to 7 mg. each, was placed in the cup. Casein was not made into pellets because it was in the form of hard wettable granules. To each nickel cup containing protein was then added 1.0 ml. of 5.0 *N* sodium hydroxide solution. The sample was mixed with the solvent and worked to remove as much occluded air as possible by means of a 23-mm. gold spatula made by flattening one end of a piece of 18-gage wire. The spatula was held firmly with a hemostat while stirring and then it was released in the cup where it remained during hydrolysis.

Filling with Hydrogen and Closing the Bomb. The nickel cup, after being charged with the hydrolysis mixture, was filled with hydrogen and closed by the following procedure, illustrated by Figure 1.

The cup was placed in the closing assembly. Cap *J*, with new lead gasket, was put in place and screw *A* was tightened to seat the gasket. *A* was then loosened, and the assembled bomb was placed in tube *B* of the hydrogen filling apparatus. Steel clip *C* was then slipped under the edge of the cap at *D* to permit ready evacuation of air and admittance of hydrogen. The hydrogen filling apparatus was made from a 55/50 standard-taper joint. *E* was a perforated cork wafer for supporting tube *B* which holds the bomb. The body of the apparatus, *F*, was supported in a clamp and the cover, *G*, was held firmly on *F* with a two-pronged clamp fastened over the top of stopcock *H*. The joint was lubricated with stopcock grease. Tube *I* was connected through a system of pinchcocks and two-way stopcocks to an aspirator and a gasometer of at least 1-liter capacity containing purified hydrogen over distilled water. The apparatus was evacuated to 35- to 40-mm. pressure and filled with hydrogen. This process was carried out three times to remove residual air. It was necessary to avoid evacuation to pressures lower than 35 mm. to prevent loss of solution by bubbling.

The apparatus was finally filled with hydrogen at a pressure of about 250 mm. of water, so that on opening the apparatus a gentle outflow of hydrogen prevented entrance of air into the bomb during the few seconds required to seal it. Stopcock *H* was closed and clip *C* was removed from under the edge of the cap with a strong magnet. Cap *J* then became seated. The apparatus was gently opened and screw *A* was quickly tightened with the fingers and then firmly tightened by turning with a case-hardened punch (a steel rod 3 mm. in diameter and 47 mm. long with a handle 8 mm. in diameter and 65 mm. long). The closed bombs were placed in a bronze spring-metal holder capable of supporting six of them in an upright position at all times.

Heating. For temperatures up to 100° C., an electrically heated oven that maintained the temperature ±2° was used. For temperatures over 100° C., an electric furnace that maintained the temperature ±3° was used.

Opening Bomb and Diluting Hydrolyzate. The bomb was cooled to room temperature and opened. If the cap were stuck, the bomb was fastened in a vise and the cap was loosened by gently tapping with a hammer the punch held upward against the rim of the cap. The cup was supported in a microbeaker, and the 23-mm. gold spatula was removed with a pair of platinum-tipped forceps. The spatula was washed with a little distilled water into a 10-ml. volumetric flask. The contents of the cup were quantitatively transferred to the volumetric flask using 1.0 ml. of 5.0 *N* sodium hydroxide and distilled water, so the solution, when diluted to volume, was 1 *N* in sodium hydroxide. A 32-mm. gold spatula was held over the edge of the cup in the usual manner as a pouring aid. The diluted hydrolyzate was filtered through a fritted-glass filter into a 25-ml. glass-stoppered Erlenmeyer flask. This solution (Solution A) was used directly for chemical tests. For the microbiological tests 2 to 6 ml. of Solution A were pipetted into a 100-ml. graduated cylinder, and distilled water was added until the volume was 90 ml. Enough 2 *N* sulfuric acid was added to neutralize the alkali of the hydrolyzate. The volume was then made up to 100 ml., and the pH was adjusted to 6.9 to 7.0 with dilute sulfuric acid or sodium hydroxide, bromothymol blue being used as indicator on a spot plate.

ANALYSIS OF HYDROLYZATES

Chemical. Tryptophan was chemically determined in hydrolyzates by an adaptation of Procedure H (*30*). To 30 mg. of *p*-dimethylaminobenzaldehyde in a 25-ml. glass-stoppered Erlenmeyer flask were added 9.0 ml. of 21.4 *N* sulfuric acid. To this solution was added 1.0 ml. of Solution A. The contents were mixed and cooled to 25° C. and allowed to stand 1 hour in the dark at 25° C. Color was then developed with 0.1 ml. of 0.04 or 0.07% sodium nitrite solution for tryptophan and proteins, respectively. Transmittancies were determined after 30 minutes. Blank solutions for protein hydrolyzates had the same composition as test solutions, except that *p*-dimethylaminobenzaldehyde was omitted. Most of the hydrolyzates gave maximum color with 0.1 ml. of the appropriate sodium nitrite solution, but some hydrolyzates of proteins or tryptophan plus added substances developed slightly more color with an additional 0.1 ml. of sodium nitrite allowed to react for 15 minutes more. This point should be determined for each hydrolyzate and the lowest transmittancy obtained converted to weight of tryptophan from curve *D*, Figure 4 (*30*).

The color of the chemical test on some protein hydrolyzates was peculiarly affected by the severity of the hydrolytic conditions used and the length of time hydrolyzates stood at 5° C. before making the test. Therefore, the characteristic behavior of each protein was determined (Table I). Casein hydrolyzates gave maximum normal blue color when hydrolyzed with maximum severity (18 hours at 151° C.) even when the test was made as soon as Solution A was prepared. Conalbumin hydrolyzates obtained by the mildest hydrolytic conditions (18 hours at 100° C.) gave an "off-colored" test and an indicated tryptophan content of 1.51% when the test was made on the same day Solution A was prepared, but the color was a normal blue and the indicated tryptophan content was 2.03% when the hydrolyzate was tested after standing 6 days at 5° C. The increase in indicated tryptophan content and return to normal color of the tests occurred after the hydrolyzates had stood at 5° for one day (Table I). This behavior appears to be related to the reversal of a reaction involving cystine which produces an interfering substance during the hydrolysis (*9*). However, the reliability of the chemical values determined on hydrolyzates that had stood one day at 5° C. is shown by their close agreement with microbiological values (Table IV).

Microbiological. Tryptophan determinations were made with *Streptococcus faecalis R.* incubated for 3 days at 30° C. The basal medium was that described by Teply and Elvehjem (*33*) for folic acid determination, except that 0.002 microgram of folic acid was added to each tube. The standard curve, for each series of tests, was made from the averaged values of duplicate determinations made over an assay range of 0 to 10 micrograms of L-tryptophan with 1-microgram increments. Tryptophan determinations on test substances were made in duplicate at five concentrations estimated to extend over the assay range of the standard curve. The tryptophan content of the test solution was taken as the average of these ten determinations. A Leitz electro-

Table I. Effect of Severity of Hydrolytic Treatment and Time of Storage of Protein Hydrolyzates at 5° C. on Color of Chemical Tests and Indicated Tryptophan Contents

Protein	Conditions of Hydrolysis: Time	Conditions of Hydrolysis: Temp.	Storage of Hydrolyzate at 5° C.	Indicated Tryptophan Content	Appearance of Test[a]
	Hours	*° C.*	*Days*	%	
Casein	18	151	0	1.19	Normal
			1	1.20	Normal
			3	1.19	Normal
			5	1.20	Normal
			10	1.20	Normal
			20	1.18	Normal
β-Lactoglobulin	18	151	0	1.17	Orchid
			1	1.73	Normal
			3	1.74	Normal
			5	1.74	Normal
			10	1.75	Normal
			20	1.74	Normal
Ox muscle	18	100	0	1.37	Normal
			7	1.37	Normal
	42	100	0	1.34	Normal
			7	1.37	Normal
			14	1.37	Normal
	18	151	0	1.22	Sl. off-colored
			6	1.34	Normal
Ovalbumin	42	100	0	1.20	Normal
			7	1.19	Normal
	18	151	0	1.01	Greenish-gunmetal
			6	1.23	Normal
Edestin	42	100	0	1.34	Normal
			7	1.35	Normal
	18	151	0	1.26	Sl. off-colored
			6	1.34	Normal
Conalbumin	18	100	0	1.51	Lavender
			6	2.03	Normal
	42	100	0	1.36	Off-colored
			7	2.03	Normal
			14	2.08	Normal

[a] Normal indicates that color was blue like that obtained with free tryptophan.

titrator was used for titration by a procedure that applied the maximum sensitivity of the instrument. The precision of the microbiological method was ±3% as determined from nine analyses (Table V).

RESULTS AND DISCUSSION

The effect of temperature on the stability of L-tryptophan in 5.0 *N* sodium hydroxide as well as the precision and accuracy of hydrolysis and analytical procedures is shown by data in Table II. Recoveries of 99.3 ± 0.4, 97.7 ± 0.6, and 96.9 ± 0.6% of the tryptophan after treatment for 18 hours at 100°, 151°, and 185° C., respectively, were obtained. Exclusion of air is essential to the success of the method. When tryptophan was similarly heated with sodium hydroxide at 100° C., except that air was left in the bomb, the recoveries were erratic and about 20 to 40% lower than were obtained using hydrogen (cf. *17*). Hydrolyzates obtained with air were yellowish and slightly turbid in contrast to clear and colorless when hydrogen was used. The nickel cups were slightly corroded when air was used but not when hydrogen was used. Recoveries of 99.3 and 96.9% of the tryptophan were obtained at 100° and 185°, respectively, with purified hydrogen as compared with recoveries of 98.2 and 96.6%, respectively, with commercial hydrogen. Although recoveries of tryptophan obtained with purified hydrogen were only slightly better than with commercial hydrogen, use of purified hydrogen was adopted to avoid the possibility of variation in purity of different lots.

The effect of time and temperature of hydrolysis of casein with 5 *N* sodium hydroxide on its indicated tryptophan content, as determined chemically and microbiologically, is shown in Table III. For calculation of results the tryptophan values found microbiologically were doubled because the organism uses only the free L form (*32*). Stokes *et al.* (*32*) showed that benzoyl DL-tryptophan was microbiologically inactive with *S. faecalis R.* and in the present work it was observed that acetyl L-tryptophan was completely inactive in supporting growth of *S. faecalis R.*

The effect of temperature on the rate of complete hydrolysis of casein is shown graphically in Figure 2, using data in Table III. The time for complete hydrolysis at each temperature was taken as that period in which the microbiological value for tryptophan content first became constant or increased by not more than 3% in an additional period of equal length. Complete hydrolysis of casein, on this basis, occurred in 2, 4, 18, 144, and 288 hours at 151°, 124°, 100°, 75°, and 50° C., respectively. When hydrolysis was complete, the microbiological method gave slightly higher tryptophan values for casein hydrolyzed at 75° and 100° C. than on hydrolyzates made at 124° and 151° C. This result may be explained by postulating incomplete racemization of the L-tryptophan on hydrolysis at the lower temperatures but complete racemization at the higher temperatures. This postulate is supported by the observation that acetyl L-tryptophan amide, which contains peptide linkages similar to those in proteins, after hydrolysis at 100° C. for 18 hours, contained 67.5% L-tryptophan instead of the 50% conforming to complete racemization. That the tryptophan values obtained by chemical and microbiological methods agreed on casein hydrolyzates made at 124° and 151° C. also supports this postulate because complete racemization of tryptophan would be more likely at the higher temperatures.

The tryptophan contents of several proteins hydrolyzed with 5 *N* sodium hydroxide at 100° for 18 and 42 hours and at 151° C. for 18 hours were determined chemically and microbiologically

Table II. Chemical Recovery of Tryptophan after Treatment with 5.0 *N* Sodium Hydroxide for 18 Hours at Various Temperatures

No. of Tests	Temperature Used: 100° C.	151° C.	185° C.	$\frac{\Sigma d}{N}$
	Average Tryptophan Recovery[a]			
	%	%	%	%
12[b]	99.3	...	...	±0.4
6[c]	98.2	...	...	±0.9
7[b]	...	97.7	...	±0.6
6[b]	...	...	96.9	±0.6
6[c]	...	...	96.6	±0.4

[a] 1.0 mg. of tryptophan used per test.
[b] Purified hydrogen used.
[c] Commercial hydrogen used.

Table III. Effect of Time and Temperature of Hydrolysis of Casein on Indicated Tryptophan Content

(As determined by chemical and microbiological analysis of hydrolyzates[a])

	Temperature Used: 50° C.		75° C.		100° C.		124° C.		151° C.	
	Chem.	Microbiol.	Chem.	Microbiol.	Chem.	Microbiol.	Chem.	Microbiol.	Chem.	Microbiol.
Hours	%	%	%	%	%	%	%	%	%	%
0.5	...	...	...	...	...	...	...	...	1.17	0.38
1	...	...	...	...	...	...	1.16	0.62	1.20	1.15
2	...	...	...	...	1.16	0.40	1.19	1.10	1.20	1.23
4	...	...	...	...	1.19	0.81	1.20	1.22	1.20	1.19
8	...	...	...	...	1.22	1.13	1.21	1.23	1.16	1.20
18	...	...	1.16	0.83	1.23	1.34	1.19	1.23	1.17	1.19
42	...	...	1.22	1.20	1.23	1.33	...	...	...	...
Days										
3	1.17	0.73	1.22	1.32	...	...	...	...	...	...
6	1.20	0.94	1.22	1.38	...	...	...	...	...	...
12	1.23	1.23	1.21	1.39	...	...	...	...	...	...
24	1.26	1.27	...	...	...	...	...	...	...	...

[a] Hydrolysis was carried out with 5.0 *N* sodium hydroxide, using 50-mg. samples of casein.

Table IV. Effect of Time and Temperature of Hydrolysis on the Tryptophan Content of Various Proteins

Protein	Temperature of Hydrolysis: 100° C., 18 Hours — Chem. %	Microbiol. %	Diff.[a] %	100° C., 42 Hours — Chem. %	Micro-biol. %	Diff.[a] %	151° C., 18 Hours — Chem. %	Micro-biol. %	Diff.[a] %
	Tryptophan Content of Proteins								
Casein	1.23	1.34	8.2	1.23	1.33	7.3	1.17	1.19	1.7
β-Lactoglobulin	...	1.83 ± 0.03[b]	...	1.74	1.79	2.2	1.75	1.84	4.9
Ovalbumin	1.16	1.32 ± 0.01[b]	12.1	1.20	1.19	0.8	1.23	1.08	13.9
Conalbumin	2.01	2.16 ± 0.01[b]	7.0	2.08	2.14	2.8	...	...	...
Edestin	1.33	1.33 ± 0.01[b]	0.0	1.34	1.41	5.0	1.31	1.28	2.3
Ox muscle	1.36	1.16 ± 0.04[b]	17.2	1.34	1.20	11.7	1.34	1.28	4.7
Arachin	0.80	0.85 ± 0.01[b]	5.9	0.83	0.87	4.6	...	...	...
CS-54R[c]	...	0.85 ± 0.00[b]	...	...	0.83	...	...	0.85	...

[a] Variation of result of chemical test from result of microbiological test.
[b] Average deviation of results obtained with two hydrolyzates.
[c] Chemical method could not be used because of interfering color which was attributed to relatively high cystine (7.4%) content of this fraction.

Table V. Racemization of L-Tryptophan by Heating with 5 *N* Sodium Hydroxide at 100° and 124° C. for Various Times[a]

Time	Temperature	No. of Tests	Tryptophan Recovery: Chemical	Microbiological[b]	Degree of Racemization[c]
Hours	° *C.*		%	%	%
18	100	9	99.3 ± 0.3	94.3 ± 3.2	11.4
68	100	1	99.3	79.2	41.6
18	124	1	97.4	72.6	54.8
41	124	2	98.2 ± 0.6	64.4 ± 3.2	71.2

[a] 1.0 mg. of L-tryptophan used per test.
[b] $\frac{\text{L-tryptophan} \times 100}{\text{DL-tryptophan}}$ = % recovery microbiologically.
[c] Calculated on basis that 50% tryptophan recovered microbiologically was equivalent to 100% racemization.

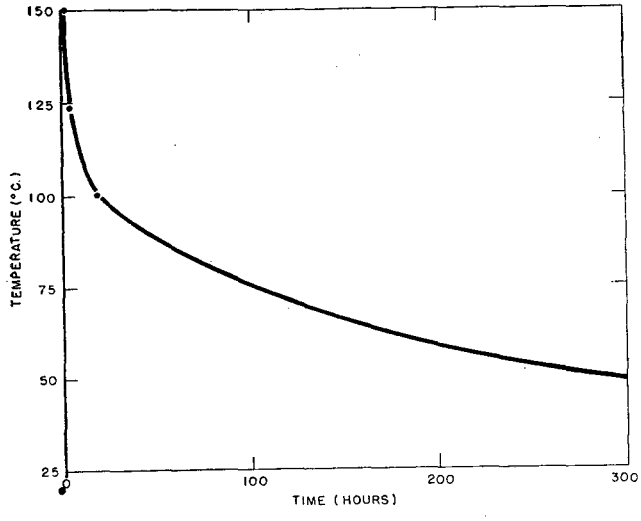


Figure 2. Effect of Temperature on Time of Complete Hydrolysis of Casein with 5 *N* Sodium Hydroxide

(Table IV). In general, the agreement between values obtained by the two methods is good and where hydrolysis had proceeded to completion the difference in values was less than 5% for every protein under one or more conditions of hydrolysis.

Although the microbiological method is an excellent one for the accurate determination of free tryptophan in protein hydrolyzates, it, as well as chemical methods requiring preliminary alkaline hydrolysis, is regarded as fundamentally unsound for the determination of tryptophan content of proteins, because of partial destruction of peptide-linked tryptophan during hydrolysis. The hydrolysis procedure described herein is important nevertheless because it provides a method which eliminates external destructive factors from consideration in studying the effects of alkali on tryptophan from proteins, free tryptophan, and tryptophan mixed with added substances, without resort to the addition of chemical protective agents which may interfere with reactions studied or the analytical procedures used. Establishment of the tryptophan contents of various proteins as determined from their alkaline hydrolyzates was regarded as essential for comparison with the values for the tryptophan contents of these same proteins by a method believed to give accurate tryptophan contents of proteins, described below.

RACEMIZATION OF L-TRYPTOPHAN AND SOME DERIVATIVES BY HEATING IN SODIUM HYDROXIDE SOLUTION

Since the work of Dakin (*5–7*) and Kossel and Weiss (*15*) it has been known that amino acids in proteins are easily racemized with alkali but free amino acids are racemized with difficulty. However, no careful determination of the conditions causing racemization of free L-tryptophan by alkali has been made, although sporadic observations have been reported (*10, 11, 16, 37*).

This study shows the effects of time and temperature on the racemization of L-tryptophan and some of its derivatives in 5 *N* sodium hydroxide under conditions whereby external destruction was prevented.

RESULTS AND DISCUSSION

Racemization was determined from results of chemical and microbiological analysis of heated alkaline solutions of tryptophan and its derivatives. The organisms use only the L form, and the chemical method determines both the D and L forms. Tryptophan and derivatives were treated by Procedure J and chemical and microbiological analysis of hydrolyzates was carried out as described above.

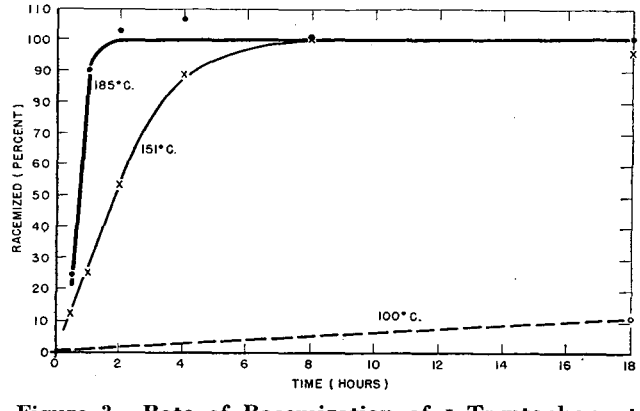


Figure 3. Rate of Racemization of L-Tryptophan at 100°, 151°, and 185° C. in 5 *N* Sodium Hydroxide

The rates of racemization of L-tryptophan when heated at various temperatures in 5 *N* sodium hydroxide are shown in Figure 3. Complete racemization occurred in 2 and 8 hours at 185° and 151° C., respectively. The degrees of racemization of L-tryptophan when heated in 5 *N* sodium hydroxide at 100° and 124° C. for various times are shown in Table V. L-Tryptophan was 11.4% racemized at 100° C. in 18 hours and 41.6% after 68

Table VI. Racemization of Derivatives of L-Tryptophan by Hydrolysis with 5 *N* Sodium Hydroxide at 100° C. for 18 Hours

Substance	No. of Tests	Sample Size	Tryptophan Recovery		Degree of Racemization[c]
			Chemical	Microbiol.[b]	
		Mg.[a]	%	%	%
L-Tryptophan	9	1.0	99.3	94.3	11.4
N-Acetyl L-tryptophan	1	1.2	98.1	85.2	29.6
N-Acetyl L-tryptophan[d]	2	1.2	98.1	81.7	36.6
N-Benzoyl L-tryptophan	1	1.5	95.9	70.5	59.0
N-Phenoxyacetyl L-tryptophan	1	1.7	98.2	86.8	26.4
p-Phenylphenacyl *N*-acetyl L-tryptophan	2	2.3	96.6	82.4	35.2
p-Phenylphenacyl *N*-phenoxacetyl L-tryptophan	1	2.6	95.9	87.0	26.0
Ethyl N-acetyl L-tryptophan	1	1.3	95.8	76.5	47.0
Ethyl L-tryptophan hydrochloride	1	1.3	98.1	94.0	12.0
N-Acetyl L-tryptophan amide	1	1.3	96.1	67.3	65.4
N-Acetyl L-tryptophan amide[d]	1	1.4	92.0	69.6	60.8

[a] Approximate quantity hydrolyzed.
[b] $\frac{\text{L-tryptophan} \times 100}{\text{DL-tryptophan}}$ = % recovered microbiologically.
[c] Calculated on basis that 50% tryptophan recovered microbiologically is equivalent to 100% racemization.
[d] Hydrolyzed 42 hours.

hours. Racemization amounted to 54.8% and 71.2% when L-tryptophan was heated at 124° C. for 18 and 41 hours, respectively.

The effects of hydrolysis with 5 *N* sodium hydroxide on the racemization of several derivatives of L-tryptophan are shown in Table VI. For interpretation of these results hydrolysis must have been complete and partial racemization of tryptophan in the preparation of derivatives must not have occurred. That hydrolysis was complete in the 18-hour period was shown because after 18 and 42 hours' hydrolysis, respectively, 85 and 82% of the total tryptophan in *N*-acetyl L-tryptophan and 67 and 70% in *N*-acetyl L-tryptophan amide were determined microbiologically as the L form. That partial racemization did not occur in the preparation of *N*-acetyl L-tryptophan was shown conclusively by du Vigneaud and Sealock (*36*) and in the preparation of the other *N*-acyl derivatives, a slight excess of alkali was maintained to prevent racemization as described by these authors (cf. *26*). The other derivatives were prepared under conditions not expected to cause racemization and all the derivatives were optically active (*28*).

No racemization was caused by hydrolysis of the ethyl ester of L-tryptophan because racemization of L-tryptophan and the ester was 11.4 and 12%, respectively. *N*-Phenoxyacetyl L-tryptophan and *N*-acetyl L-tryptophan showed about the same degree of racemization—26.4 and 29.6%, respectively. But racemization of *N*-benzoyl L-tryptophan was 59% or twice as great as that of the former two compounds. According to Levene and Steiger (*18*) the extent of racemization of ketopiperazines increased with their stability toward hydrolysis. The greater degree of racemization of benzoyl derivative, therefore, may be due to greater resistance to hydrolysis as compared to the acetyl and phenoxyacetyl derivatives. The *p*-phenylphenacyl esters of *N*-acetyl and *N*-phenoxyacetyl L-tryptophan showed no significant increase in racemization as compared with the corresponding acidic derivatives because 35.2 and 26.0% racemization, respectively, were found for the esters, and 29.6 and 26.4%, respectively, for the *N*-acyl derivatives. In contrast, however, the ethyl ester of *N*-acetyl L-tryptophan was 47% racemized on hydrolysis as compared to 29.6% for *N*-acetyl L-tryptophan. The racemization (65.4%) obtained with *N*-acetyl L-tryptophan amide was greater than that obtained with any of the other derivatives, as was anticipated because the two amide groups were expected to be most resistant to hydrolysis.

Dakin (*5–7*) recognized that the relative ease of racemization of derivatives of amino acids was influenced by factors other than the ability to form enol salts, but did not make it clear from his theory why the *N*-acyl derivatives of amino acids containing a free carboxyl group should racemize more readily than the free amino acids. Yet the degree of racemization of *N*-acetyl L-tryptophan was 2.5 times and that of *N*-benzoyl L-tryptophan was 5 times greater than that of free L-tryptophan. These results show the need for extension of Dakin's enol theory.

Significantly higher values for the tryptophan content of casein were obtained by the microbiological method on complete hydrolyzates prepared at 75° and 100° C. than on complete hydrolyzates made at 124° and 150° C. These results were explained by postulating incomplete racemization of tryptophan when casein was hydrolyzed at 100° and 75° C. According to Dakin's theory, this incomplete racemization might be attributed to the presence of some terminal tryptophan with free carboxyl groups, but the results of the present study indicate that some tryptophan even with carboxyl groups in peptide linkage might be hydrolyzed at the lower temperatures without racemization.

EFFECT OF AMINO ACIDS AND CARBOHYDRATES ON TRYPTOPHAN HEATED IN SODIUM HYDROXIDE SOLUTION

The effect of amino acids and carbohydrates on tryptophan when heated in 5 *N* sodium hydroxide was determined in an attempt to elucidate the causes of destruction of tryptophan during alkaline hydrolysis of proteins. Procedure J was used and chemical and microbiological analysis of hydrolyzates was carried out as described above. As cystine, cysteine, and lanthionine caused interference with the colorimetric analysis of hydrolyzates, the microbiological method was used with these amino acids. Graham *et al.* (*9*) observed a similar interference.

RESULTS

The effect of sixteen amino acids on L-tryptophan when heated in 5 *N* sodium hydroxide at 150° C. for 18 hours is shown in Table VII. The molar ratio of amino acid to tryptophan was approximately 4 in each test. Alanine, β-phenylalanine, tyrosine, leucine, glycine, glutamic acid, proline, hydroxyproline, arginine, lysine, histidine, and methionine caused no destruction of tryptophan; recoveries of trypotophan from 97 to 99% were obtained. Cystine, serine, and threonine, however, caused 23.7, 23.9, and 21.7% destruction of tryptophan, respectively.

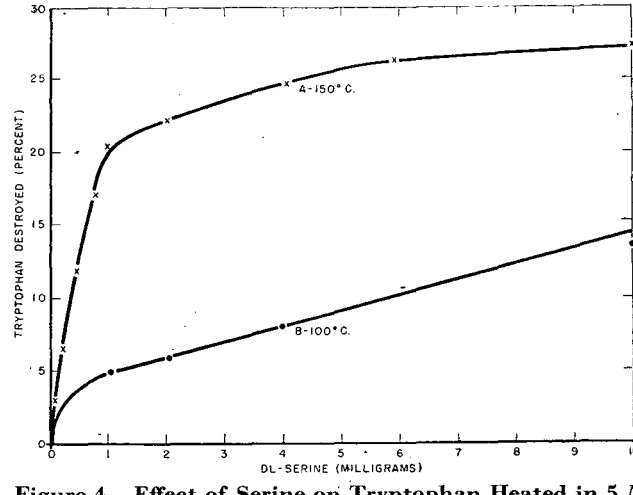


Figure 4. Effect of Serine on Tryptophan Heated in 5 *N* Sodium Hydroxide for 18 Hours at 100° and 150° C.

Table VII. Effect of Amino Acids on Tryptophan Heated in 5 *N* Sodium Hydroxide for 18 Hours at 150° C.[a]

Amino Acid		Molar Ratio Amino Acid/ Tryptophan	Tryptophan Recovered[b]
	Mg.		%
DL-Alanine	1.81	4.1	99.0
DL-β-Phenylalanine	3.36	4.2	98.6
L-Tyrosine	3.74	4.2	97.0
L-Leucine	2.63	4.1	98.6
Glycine	1.61	4.4	97.4
L-Glutamic acid	2.95	4.1	98.6
L-Proline	2.36	4.2	99.0
L-Hydroxyproline	2.54	4.0	98.2
L-Arginine hydrochloride	4.21	4.1	99.4
L-Lysine hydrochloride	3.73	4.2	99.4
L-Histidine dihydrochloride	4.66	4.2	99.3
DL-Methionine	3.04	4.2	97.0
L-Cystine	4.81	4.0	76.3
meso-Lanthionine[c]	4.18	4.1	
DL-Serine	2.07	4.0	76.1
DL-Threonine	2.51	4.3	78.3

[a] Procedure. To the indicated weight of amino acid in the nickel cup of the bomb was added 1.0 ml. of a freshly prepared 5 *N* sodium hydroxide solution containing 1.00 mg. of L-tryptophan. Procedure J was then used.
[b] Tryptophan in the unheated 5 *N* sodium hydroxide solution was considered 100%. In control hydrolyses, recoveries of tryptophan hydrolyzed alone were always near the 97.7 ± 0.6% value reported for this method.
[c] Not determined because of interfering color.

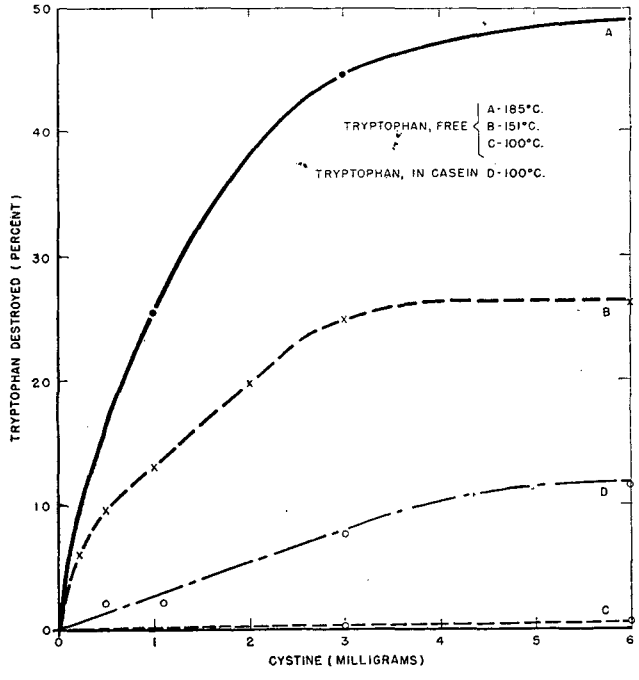


Figure 5. Effect of Cystine on Tryptophan, Free and in Casein, Heated for 18 Hours at Various Temperatures

The destructive effect of various quantities of serine when heated in 5 *N* sodium hydroxide for 18 hours at 100° and 150° C. with 1 mg. of tryptophan is shown by curves *B* and *A* (Figure 4). The destructive effect of serine as shown by the chemical test was confirmed by microbiological analysis. It is apparent that serine, unlike cystine, destroys some free tryptophan in alkaline solution under nonracemizing conditions.

The effect of cystine on L-tryptophan when heated together in 5 *N* sodium hydroxide at temperatures that cause or do not cause racemization is shown in Figure 5. When 1 mg. of cystine and 1 mg. of L-tryptophan were heated together in 1 ml. of 5 *N* sodium hydroxide for 18 hours at 185° and 151° C., the destruction of L-tryptophan was 25 and 13%, respectively (curves *A* and *B*). The amount of destruction increased with the quantity of cystine until with a sixfold ratio of cystine to L-tryptophan about half of the tryptophan was destroyed at 185° and about a quarter at 151° C. However, when 1 mg. of L-tryptophan was similarly heated with 6 mg. of cystine at 100° C. the amount of destruction was negligible (curve C). The lack of destruction at 100° C. might be attributed to a temperature effect and not to lack of racemization of tryptophan.

This point was settled by taking advantage of the fact that practically complete racemization of peptide-linked tryptophan occurs at 100° C. on hydrolysis of a protein with sodium hydroxide. Casein was chosen because it has a cystine content relatively lower than other proteins, and therefore the destructive effect of added cystine should be more easily detected. As shown in Figure 5, curve *D*, 12% of the tryptophan was destroyed when 50 mg. of casein and 6 mg. of cystine were hydrolyzed for 18 hours at 100° C. with 5 *N* sodium hydroxide. Thus, tryptophan is destroyed at 100° C. under racemizing conditions and not destroyed under nonracemizing conditions because the 12% destruction of tryptophan from casein is four times the experimental error of the microbiological analysis. It was shown experimentally that heating cystine alone with alkali did not produce anything that inhibited *S. faecalis R.* Thus, 6 mg. of cystine were heated at 185° for 18 hours in 1 ml. of 5 *N* sodium hydroxide. A quantity of this hydrolyzate equal to that used in the tests was added to a control solution of tryptophan and a 100% recovery of tryptophan was obtained microbiologically.

Cysteine has approximately the same destructive effect on tryptophan as cystine. The destructive effects of cystine and cysteine were the same on both L- and DL-tryptophan, as would be anticipated.

Because of the destructive effect of cystine on tryptophan in casein in alkaline solution at 100° C. it was of interest whether or not similar destruction of tryptophan occurred on hydrolysis of derivatives of tryptophan. Results in Table VIII show that the destructive effect of cystine was not significant with *N*-acetyl L-tryptophan and *N*-acetyl L-tryptophan amide and was barely significant with *p*-phenylphenacyl *N*-acetyl L-tryptophan. The degrees of racemization of these compounds under conditions of the test were 30, 65, and 35%, respectively (Table VI), whereas racemization of tryptophan from casein under these conditions is almost 100%. These results, although inconclusive, indicate that factors other than racemization alone may be involved in the destruction of tryptophan in proteins by cystine during alkaline hydrolysis at 100° C. Dakin concluded that not only simple ability to enolize but probably the nature of the groups attached to the amino and carboxyl groups were involved in the relatively easy racemization of amino acids on hydrolysis of proteins with alkali. Similar factors may account for differences in the effect of cystine on tryptophan in complex proteins as compared to the simple derivatives.

Table VIII. Effect of Cystine on Some Derivatives of L-Tryptophan Heated in 5 *N* Sodium Hydroxide for 18 Hours at 100° C.

Tryptophan Derivative	Weight per Test[a]	Cystine Added	Tryptophan Recovered[b]
	Mg.	*Mg.*	%
Acetyl L-tryptophan	1.21	0	100
		3.1	98.9
		6.0	98.4
p-Phenylphenacyl *N*-acetyl L-tryptophan	2.29	0	100
	2.32	0.5	102
	2.29	1.1	98.1
	2.28	2.1	97.5
	2.28	4.0	96.0
N-Acetyl L-tryptophan amide	1.34	0	100
	1.28	3.1	98.6
	1.33	6.0	99.3

[a] 1 ml. of sodium hydroxide used for each sample.
[b] Determined microbiologically. L-tryptophan determined microbiologically, without added cystine, was regarded as 100% recovery.

Kuiken *et al.* (*17*) recently reported that cysteine, cystine, and methionine protected tryptophan from oxidation during hydrolysis with alkali. Probably they did not observe the destructive effects of cystine on free tryptophan because they autoclaved, presumably at 125° C., with 4 *N* sodium hydroxide for 8 hours. According to an estimate based on data in Table V, racemization under these conditions would have been approximately 15%. This amount of racemization is approximately equivalent to that obtained under the conditions used to obtain curve *C* (Figure 5) where no destruction of tryptophan by cystine was obtained. Results of this study, therefore, are not incompatible with Kuiken's observations.

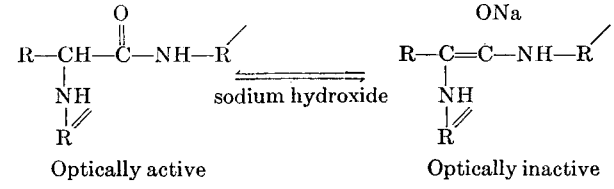
The effect of glucose and fructose on tryptophan when heated in 5 *N* sodium hydroxide for 18 hours at 150° C. was determined. The molar ratio of carbohydrate to tryptophan was approximately 4 in each test. The alkaline solutions were clear and colorless after the hydrolytic treatment. Recoveries of 96.2 ± 0.2 and 95.9 ± 0.2% of the tryptophan were obtained with glucose and fructose, respectively, using colorimetric analysis and there was no interference with the color. When alkaline hydrolyzates were analyzed microbiologically using *S. faecalis R.*, 93.7 ± 0.7 and 97.1 ± 0.9%, respectively, of the tryptophan shown to be present colorimetrically was found. It was concluded that, under the conditions of the test, these carbohydrates have an almost negligible destructive effect on tryptophan because the recovery of tryptophan heated alone under the same conditions was 97.7 ± 0.6%.

The protective effect of nine amino acids against destruction of tryptophan by serine when heated in 5 *N* sodium hydroxide for 18 hours at 150° C. was determined, using quantities of each amino acid large enough to approximate the proportions of amino acids in a protein. A weight ratio of amino acid to tryptophan of 50 and a molar ratio of serine to tryptophan of approximately 4 were chosen to simulate a hypothetical protein containing 2% tryptophan and 4% serine. Results of this experiment using arginine, lysine, histidine, proline, hydroxyproline, glutamic acid, glycine, alanine, and tyrosine, are shown in Table IX. Column 4 shows the recoveries of tryptophan in the presence of these amino acids both with and without serine. The recoveries of tryptophan in the presence of seven of the amino acids, without serine present, varied within 1.9% greater to 0.6% less than the average recovery of tryptophan when heated alone under the same conditions. Histidine dihydrochloride caused an *apparent increase* of 5.2% and tyrosine a loss of 6% of the tryptophan. The degrees of protection of tryptophan against destruction by serine varied from practically complete with histidine and hydroxyproline to almost none with proline as shown in column 7, Table IX.

Proteins also have a protective effect against destruction of tryptophan by serine on hydrolysis with 5 *N* sodium hydroxide for 18 hours at 100° C. Thus 7 mg. of serine heated with 50-mg. samples of casein, edestin, ovalbumin, and ox muscle had no effect on their tryptophan contents.

DISCUSSION

Belief that proteins can be hydrolyzed with alkaline agents without significant destruction of tryptophan originated from observations that free tryptophan is relatively stable under the conditions of hydrolysis and that good recoveries of tryptophan added to proteins before hydrolysis have been obtained. This concept is based on the assumption that free tryptophan and peptide-linked tryptophan have the same stabilities under these conditions. Demonstration that some tryptophan is destroyed by cystine in alkaline solution at 100° C. under racemizing conditions but that none is destroyed under nonracemizing conditions shows the fallacy of this deduction. That free and peptide-linked tryptophan should have different stabilities in alkaline solution is to be expected from consideration of Dakin's (*5–7*) work. He first demonstrated that amino acids in peptide linkage enolized in alkaline solution as follows:



Optically active — Optically inactive

The free amino acids do not enolize under these conditions. It is probable, therefore, that tryptophan in the enol form is less stable than in the unenolized form because of the presence of the double bond.

The effect of the amino acids on tryptophan in 5 *N* sodium hydroxide was determined by heating at 150° C. for 18 hours because free tryptophan is completely racemized under these conditions. This provides conditions for evaluating the destructive effects on free tryptophan more comparable with those of peptide-linked tryptophan during hydrolysis of proteins at lower temperatures but where racemization of tryptophan occurs more easily than with free tryptophan.

Some generalizations can be made regarding the relationship of structure and functional groupings of amino acids to their effect on free tryptophan in sodium hydroxide solution. The results are influenced in some cases by the relative proportions of amino acid to tryptophan and data shown in Tables VII and IX were obtained using a molar ratio of amino acid to tryptophan of 4 (lower concentration) and a weight ratio of amino acid to tryptophan of 50 (higher concentration).

Table IX. Effect of Amino Acids in Preventing Destruction of Tryptophan by Serine Heated in 5 *N* Sodium Hydroxide for 18 Hours at 150° C.[a]

Amino Acid		DL-Serine	Tryptophan			
			Recovered[b]	Increase or decrease from av. of tryptophan alone[c]	Destroyed by serine[d]	Degree of protection from destruction by serine[e]
	Mg.	*Mg.*	%	%	%	%
L-Arginine hydrochloride	59.0	0	99.3	+1.6	3.2	85.8
L-Arginine hydrochloride	59.0	2.20	96.1			
DL-Lysine hydrochloride	63.0	0	99.6	+1.9	3.5	86.5
DL-Lysine hydrochloride	63.0	2.26	96.1			
L-Histidine dihydrochloride	74.0	0	102.9	+5.2	0.4	98.2
L-Histidine dihydrochloride	74.4	2.21	102.3			
L-Proline	50.0	0	96.5	−1.2	21.1	5.2
L-Proline	49.9	2.19	75.4			
L-Hydroxyproline	50.0	0	97.8	+0.1	+ 1.2	105.0
L-Hydroxyproline	50.0	2.17	99.0			
L-Glutamic acid	50.1	0	97.1	−0.6	8.6	62.1
L-Glutamic acid	50.1	2.34	88.5			
Glycine	50.2	0	96.7	−1.0	2.1	90.6
Glycine	50.0	2.15	94.6			
DL-Alanine	50.0	0	96.8	−0.9	8.3	63.1
DL-Alanine	50.1	2.18	88.5			
L-Tyrosine	50.0	0	91.7	−6.0	13.2	41.6
L-Tyrosine	50.1	2.26	78.5			

[a] Procedure. To the indicated weight of substance in the cup of the bomb was added 1.0 ml. of a 5 *N* sodium hydroxide solution containing 1.0 mg. of tryptophan. The contents were stirred with the gold spatula and treated by Procedure J.

[b] Recoveries were calculated considering the tryptophan in the original unheated tryptophan solution as 100%.

[c] Average recovery of tryptophan alone was 97.7 ± 0.6%.

[d] Difference between tryptophan recovered without and with serine present.

[e] $100 - \frac{\%\text{ tryptophan destroyed by serine in presence of amino acid}}{\%\text{ tryptophan destroyed by serine alone, from curve } A\text{, Figure 4}} \times 100$ equals degree of protection.

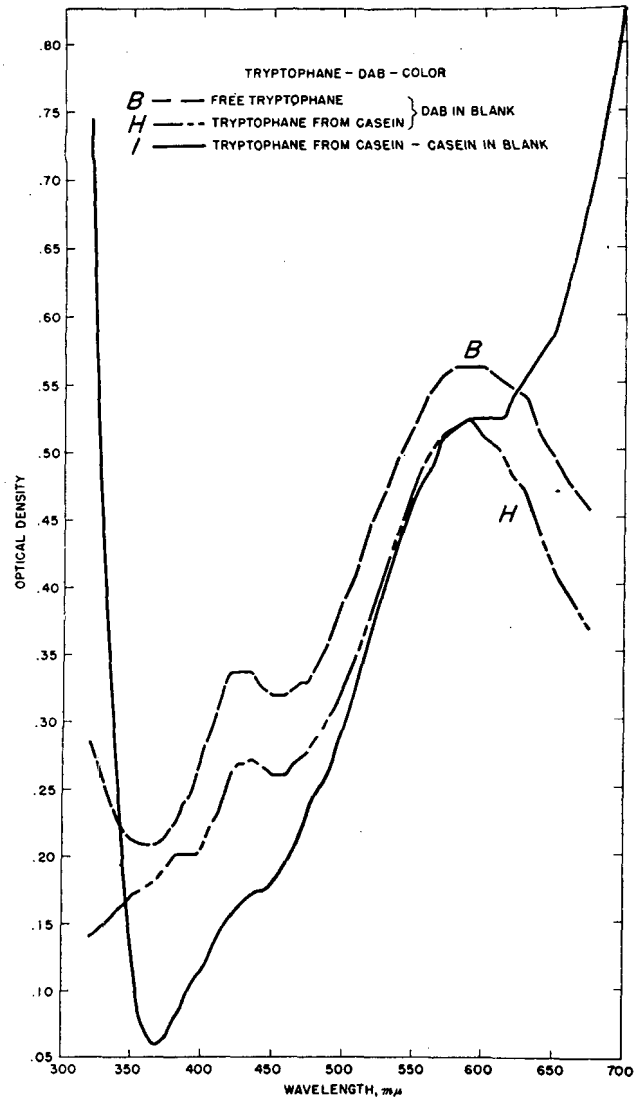


Figure 6. Comparison of Absorption Curves of Tryptophan-*p*-Dimethylaminobenzaldehyde Color from Free Tryptophan and Tryptophan from Casein

***B*. Free tryptophan. Color developed by Procedure C (*30*) using 100 micrograms of tryptophan per test. Time of Reaction I was 24 hours. *H, I*. Tryptophan from casein. Color developed by Procedure K using 6 mg. of casein per test. Time of Reaction I was 24 hours. *H*. *p*-Dimethylaminobenzaldehyde in blank. *I*. Casein in blank**

Compounds having SH groups or groups convertible to SH, such as cystine, cysteine, and lanthionine, destroy some tryptophan when heated under racemizing conditions. The CH_3S group of methionine did not destroy tryptophan in the lower concentration but produced an interfering color and apparently destroyed some of it at the higher concentration under racemizing conditions.

The effect of the hydroxyl group depends on its structural relationship to other functional groups in the molecule. When α to the amino group and β to the carboxyl group, as in serine and threonine, the hydroxyl group destroys tryptophan under either racemizing or nonracemizing conditions. The hydroxyl group of hydroxyproline β to the immino group and γ to the carboxyl group has no destructive effect on tryptophan even at the higher concentration under racemizing conditions. The phenolic hydroxyl of tyrosine has no destructive effect at the lower concentration and only relatively slight destructive effect at the higher concentration. The polyhydroxy compounds, glucose and fructose, had negligible destructive effects at the lower concentration.

The guanidino group of arginine, the imidazol group of histidine, the ϵ-amino group of lysine, and the carboxylic group of glutamic acid were without destructive effects.

The relation of structure and nature of functional groupings of the amino acids to their effectiveness in protecting tryptophan from destruction by serine is somewhat obscure. The protective effect of the α-aminocarboxyl grouping is influenced by relatively slight differences in the rest of the molecule—for example, glycine was 90.6% effective in protecting tryptophan from serine, whereas alanine was only 63.1% effective. The tryptophan-protective effect of the hydroxyl group is more surprising because hydroxyproline had an indicated protective effect of 105% whereas proline gave only 5% protection against destruction by serine. Tyrosine had an intermediate protective effect of 41.6%. Of the basic amino acids histidine gave complete protection whereas arginine and lysine were 85.8 and 86.5% effective, respectively. The peptide-linked amino acids of the proteins, casein, edestin, ovalbumin, and ox muscle were completely effective in preventing destruction of tryptophan by added serine.

The destructive action of cystine, cysteine, lanthionine, serine, and threonine in tryptophan in alkaline solution is probably one of oxidation. But explanation of the chemistry of the protective action of various amino acids in preventing this oxidation must await further experimentation.

DETERMINATION OF TRYPTOPHAN IN PROTEINS

Cystine, cysteine, lanthionine, serine, and threonine cause significant destruction of tryptophan on heating in alkaline solution, but none of these amino acids has any destructive effect on tryptophan in sulfuric acid solution under the conditions used for the determination of tryptophan starting with the unhydrolyzed protein. Therefore, the optimum conditions for the determination of tryptophan in proteins starting with the intact protein were determined and evidence for the accuracy of the methods developed has been evaluated critically.

The reactions involved in the determination of tryptophan in proteins, like those in the determination of free tryptophan (*30*), were studied in two steps. Reaction I was the combination of tryptophan and *p*-dimethylaminobenzaldehyde in sulfuric acid solution to form a colorless condensation product, and Reaction II was the development of the blue color by oxidation with sodium nitrite.

Casein was used to determine optimum conditions for the reactions in preliminary studies, and the methods were then generalized on the basis of studies with several proteins.

CONCENTRATION OF SULFURIC ACID FOR REACTION I

Maximum color with free tryptophan was obtained when condensation with *p*-dimethylaminobenzaldehyde took place in 12 to 13.2 *N* sulfuric acid (throughout this paper the term acid refers to sulfuric acid) (*30*). But 13 *N* acid was not concentrated enough for a general method of tryptophan analysis starting with the intact proteins because precipitates sometimes formed in test solutions and dissolved very slowly or never completely dissolved. Furthermore, Reaction I required more than 48 hours to give maximum color with some proteins. Therefore, the maximum concentration of acid which could be used for Reaction I was determined. When 22 *N* acid was used in tests with free tryptophan some charring took place; but when 19 *N* acid was used the tryptophan–*p*-dimethylaminobenzaldehyde complex was stable for 72 hours at 25° C. (Table VI, *30*). All the proteins studied dissolved in 19 *N* acid to give clear solutions, and maximum color was obtained in all cases when Reaction I proceeded from 3 to 24 hours. Therefore, 19 *N* acid was adopted for the determination of tryptophan in proteins. Tillmans and Alt (*34*) stated that the intensity of the tryptophan color obtained with formaldehyde was independent of the acid concentration between the limits of 60 to 70 weight % and they used 66 to 67% (21.4 *N*) as preferable

Table X. Summary of Kinetic Studies of Reaction II with Nine Proteins[a]

Protein	Weight per Test	Sodium Nitrite Used to Produce Maximum Color: Calcd. molar ratio[b]	Sodium Nitrite Used to Produce Maximum Color:	Time to Produce Maximum Color	Time, Minutes: 0	1	3	5	7	10	15	30	60	120	180	1440
	Mg.		%[c]	*Min.*	% Maximum Color Formed[d]											
Casein	6.0	1.4	0.045	30–60	10	81	92	96	97	99	99.5	100	100	99.5	100	95
Ovalbumin	6.0	2.0	0.060	60	10	58	74	81	87	91	94	98	100	99	97	93
Conalbumin	3.0	1.1	0.030	15–30	13	82	92	95	97	98	100	100	99	98	98	93
β-Lactoglobulin	4.5	1.3	0.045	30	9.4	75	90	95	98	99	99.5	100	99.5	99	98	92
Edestin	5.5	1.6	0.045	15–30	15	83	93	96	98	99	100	100	99.8	99	98	91
Arachin	8.5	1.1	0.030	30	32	85	94	96	99	99.6	99.6	100	97	96	96	87
Ox muscle	7.0	1.7	0.060	30	11	76	87	92	94	97	98	100	99.5	99	98	92
CS-54R[e]	8.0	1.2	0.030	7–15	18	87	97	99.7	100	100	100	99	97	94	92	86
CS-56R[e]	11.6	1.3	0.030	15	17	83	94	97	99	99.7	100	98	97	94	91	84
Free tryptophan	0.10	1.0	0.034	30–60	7	75	87	90	93	96	96	100	100	...	100	90

[a] A standardized procedure similar to that described for casein was used for all proteins. To 58.356 mg. of casein (6.0 mg. per test) and 292 mg. of *p*-dimethylaminobenzaldehyde (30 mg. per test) in a 125-ml. glass-stoppered Erlenmeyer flask were added 97.3 ml. of 19 *N* acid at 25° C. The suspension was placed in the dark at 25° C. and shaken occasionally. After 24 hours' standing, 10-ml. aliquots were removed and used for each test. Transmittancy was determined and then 0.1 ml. of sodium nitrite of the desired concentration was added and the solution shaken. Transmittancies at 590 mμ were then determined at the indicated intervals. Kinetic studies using 0.1 ml. of 0.015, 0.030, 0.045, 0.060, 0.090, and 0.12% sodium nitrite per test were made on each protein, but only a concentration which produced maximum color is reported in this table.

[b] Molar ratio of sodium nitrite to tryptophan. The weight of tryptophan per test was estimated from the minimum transmittancy reading obtained in each series of tests.

[c] Concentration of sodium nitrite added to each test in 0.1 ml. of water solution.

[d] Transmittancies were converted to weight of tryptophan from curve *C*, Figure 4 (*30*), and then expressed in this table as % of maximum weight found in a given series of tests.

[e] When this protein, *p*-dimethylaminobenzaldehyde, and sulfuric acid were mixed the solution acquired a bright pink color which was more intense and more permanent than that formed with most proteins. With most of the proteins the initial pink color faded in 5 to 15 minutes. The pink color with this protein remained undiminished for 2 hours and required 5 hours to become colorless.

Table XI. Rates of Reaction I Using Proteins in Solution[a]

Protein	Mg. per Test	Solvent[b]	Time, Minutes: 1	5	15	30	60	120	180	240	360	600	Time, Days: 1	2	3	5
			% Tryptophan Reacted[c]													
Casein	6.00	A	58	68	77	83	89	95	97	97	98	..	100	98	97	95
Ovalbumin, D	6.00	A	59	65	73	80	87	92	96	96	...	..	100	99.3	96	91
Ovalbumin, N	6.05	W	66	73	79	85	93	97	100	100	99.7	..	96	93	88	80
Conalbumin, N	3.19	W	57	67	77	84	93	97	99.3	100	100	97	89	80	..	..
β-Lactoglobulin	4.50	A	50	60	70	81	90	96	100	98	97	..	87	86	83	82
Edestin	5.50	A	63	70	79	85	91	97	99	100	100	..	97	93	90	85
Arachin	8.50	A	64	70	78	83	91	96	97	100	100	..	96	93	89	85
CS-13R1[d]	10.1	W	49	64	74	83	89	95	97	98	98	..	100	98	96	95

[a] Procedure K was used except that the indicated times of Reaction I were used.

[b] A represents 0.1 *N* sodium hydroxide solution; W represents water.

[c] Weight of tryptophan equivalent to lowest transmittancy obtained for a given protein was considered 100%.

[d] See footnote[e], Table X.

starting concentration although the concentration after addition of the tryptophan-containing solution would be lower than 21.4 *N*.

CHOICE OF WAVE LENGTH AND CONFORMITY TO BEER'S LAW

The wave length–density relationships of the tryptophan–*p*-dimethylaminobenzaldehyde colored complex using free tryptophan and tryptophan from casein are compared in Figure 6, curves *B* and *H*, respectively. The general shape of the curves corresponds closely from 400 to 690 mμ and both have well-defined absorption bands in the spectral region of 425 and 590 mμ.

The effect of the composition of the blank solution on the wave length–density relationships of casein-tryptophan color is shown by comparing curves *H* and *I*. *I* was obtained using a blank solution containing a quantitity of casein equal to that used in the test but no *p*-dimethylaminobenzaldehyde, and *H* was obtained with the same test solution using a blank solution which contained *p*-dimethylaminobenzaldehyde but no casein. The relationship between curves *H* and *I* is similar to that between curves *E* and *F* (Figure 2, *30*), in which the free tryptophan–*p*-dimethylaminobenzaldehyde color was measured using blank solutions containing *p*-dimethylaminobenzaldehyde and free tryptophan, respectively. Like *E* and *F*, *H* and *I* coincided at the critical wave lengths near 590 mμ. This coincidence is important in the analysis of proteins because test solutions may contain a slight amount of adventitious color, the effect of which can be eliminated by using a blank solution containing protein but no *p*-dimethylaminobenzaldehyde.

Curves *H* and *I* were obtained with a Beckman quartz spectrophotometer, but a Coleman Model 11 spectrophotometer was used for the rest of the investigation; so results obtained with two instruments were compared. The Beckman instrument gave a plateau of maximum absorption from 590 to 615 mμ with casein in the blank, but with *p*-dimethylaminobenzaldehyde in the blank an equal degree of maximum absorption occurred sharply at 590 mμ. The Coleman instrument, however, gave a plateau of maximum absorption extending from 570 to 600 mμ with *p*-dimethylaminobenzaldehyde in the blank, but with casein in the blank the same degree of maximum absorption occurred sharply at 590 mμ. Absorption decreased, after passing the maximum, with increasing wave length when either casein or *p*-dimethylaminobenzaldehyde was in the blank with the Coleman instrument. But with the Beckman instrument, absorption increased with higher wave lengths after passing 590 mμ when casein was in the blank but decreased when *p*-dimethylaminobenzaldehyde was in the blank. However, with both instruments and with both blanks, maximum absorption or a distinct plateau occurred in the region of 590 mμ. Other proteins, using the Coleman instrument, gave maximum absorption, with protein in the blank, at wave lengths from 580 to 620 mμ.

Differences in tryptophan values of nine representative proteins when calculated from the transmittancy obtained at 590 mμ or from the minimum transmittancy over the wave-length range of 580 to 620 mμ were determined. The average difference in values was only 1.7%, four proteins showed no difference, and the maximum difference was 6%. Whether it is more accurate to use uniformly the transmittancy obtained at 590 mμ or the minimum transmittancy obtained over the wave-length range of 580 to 620 mμ to calculate results cannot be definitely stated. However, the later procedure gave average recoveries accurate to 0.7% with several pure derivatives of tryptophan; therefore, the transmittancy of minimum wave length over the range of 580 to 620 mμ was used in this work.

The results obtained with casein showed excellent conformity to Beer's law. A straight line passing through the origin was

obtained when the log per cent transmittancy was plotted against weight of casein using samples from 1 to 7 mg. per test and representing transmittancies ranging from 73 to 12%, respectively.

OPTIMUM CONCENTRATION OF *p*-DIMETHYLAMINOBENZALDEHYDE

The optimum concentration of *p*-dimethylaminobenzaldehyde for analysis of proteins was determined using quantities of *p*-dimethylaminobenzaldehyde ranging from 1 to 100 mg. per test with 3 and 6 mg. of casein per test. Results showed that 10, 20, 30, and 40 mg. and 20 and 30 mg. of *p*-dimethylaminobenzaldehyde per test gave maximum color with 3 and 6.0 mg. of casein, respectively. Thirty milligrams of *p*-dimethylaminobenzaldehyde per test were adopted as optimum for the determination of tryptophan in proteins as for determination of free tryptophan (*30*).

OPTIMUM CONDITIONS FOR REACTION II USING PROTEINS

The time and concentration of sodium nitrite required to produce maximum color were determined using nine proteins. Molar ratios of sodium nitrite to tryptophan of approximately 0.5, 1, 1.5, 2, 3, and 4 were used and this range always revealed one or more quantities of sodium nitrite which gave maximum color. The relative maximum color intensities with casein, for example, were 97, 100, 100, 100, 99, and 97% using molar ratios of sodium nitrite to tryptophan of 0.45, 0.90, 1.4, 1.8, 2.7, and 3.6, respectively. Table X contains data on the rate of Reaction II for one quantity of sodium nitrite which gave maximum color for each protein. The optimum molar ratios varied from 1.1 with conalbumin and arachin to 2.0 with ovalbumin. The optimum ratio for free tryptophan was 1 to 1.5. The optimum times for Reaction II varied from 7 to 15 minutes for the natural proteose fraction CS 54R to 60 minutes for ovalbumin. In most cases the intensity of the color decreased only slightly in 3 hours. The quantity of protein used per test varied from 3 to 11.6 mg., depending on its tryptophan content.

The results show that to obtain maximum color intensity in the determination of tryptophan in proteins, it is necessary to determine the optimum quantity of sodium nitrite and the optimum time of Reaction II for each protein. However, from the available data a constant time and a constant quantity of sodium nitrite were chosen which gave a tryptophan value with a minimum deviation from that obtained using optimum conditions for each protein. The composite optimum conditions, thereby, chosen for Reaction II were: 0.1 ml. of 0.045% sodium nitrite per test and a reaction time of 30 minutes. The tryptophan contents of each protein, as determined using individual and composite optimum conditions for Reaction II, were compared. Identical values were obtained by both methods with free tryptophan, casein, β-lactoglobulin, and edestin. The differences in values obtained with other proteins were arachin, 0.7%; ox muscle, 1.4%; conalbumin, 1.6%; CS-54R, 2.3%; ovalbumin, 2.5%; and CS-56R, 2.9%. The average difference obtained with individual and composite optimum conditions for Reaction II for the nine proteins was 1.3%, which is regarded as typical of the error likely to occur with other proteins. However, in analyzing new protein preparations it is recommended that the optimum conditions for Reaction II be determined if greatest accuracy is desired.

RATE OF REACTION I WITH PROTEINS AND STABILITY OF TRYPTOPHAN-*p*-DIMETHYLAMINOBENZALDEHYDE CONDENSATION PRODUCT AT 25° C.

The rates of Reaction I in 19 *N* acid at 25° C. were studied with several proteins. This study was based on the observation, also made with free tryptophan, that Reaction I is prevented if sodium nitrite is added to the acid solution of *p*-dimethylaminobenzaldehyde just before the addition of the protein. If sodium nitrite is added at any time before Reaction I is completed, no appreciable further condensation occurs but color is formed by oxidation of that portion of the tryptophan and *p*-dimethylaminobenzaldehyde already reacted.

To study the rate of Reaction I in short-time intervals, proteins dissolved in water or dilute sodium hydroxide were added to the test solutions because many proteins, when added in the solid form, required several hours for complete solution. The time required for Reaction I to give maximum color by Procedure K (Procedures K to P for determination of tryptophan in proteins are described below) varied with different proteins from 3 hours with β-lactoglobulin and native ovalbumin to 24 hours for casein, denatured ovalbumin, and a cottonseed allergenic protein fraction CS-13R1 (similar to CS-56R) (Table XI). However, the rates of the early stages of Reaction I for the different proteins were uniform. Thus, in 15, 30, and 60 minutes the average percentage completion of Reaction I for the eight proteins was 75.5 ± 2.8, 82.9 ± 1.5, and 90.6 ± 1.7%, respectively

Slight destruction of tryptophan occurs on solution of proteins in dilute sodium hydroxide. Therefore, a general method of tryptophan analysis of proteins was developed in which contact with alkali was avoided. Three procedures, N, O, and P, were developed in which 19 *N* acid was added directly to the solid protein and solid *p*-dimethylaminobenzaldehyde. In Procedure N individual tests consisting of protein sample, *p*-dimethylaminobenzaldehyde (30 mg.), and 10 ml. of 19 *N* acid were set up and color was developed at desired intervals. Procedure O was used to prepare several tests by removing aliquots from a single large sample—for example, in the determination of optimum conditions for Reaction II. When Procedure O was used slight errors in-

Table XII. Rates of Reaction I Using Proteins in Solid State

Protein	Procedure[a]	Mg. of Sample	19 *N* Acid, Ml.	Hours Required for Solution of Protein	Time of Reaction I, Hours: 1	2	3	4	5	6	7	8	9	10	12	24	48
					Tryptophan Content, %												
β-Lactoglobulin	O	60.07	200	3 to 4	...	...	...	2.40	2.42	2.43	2.41	2.39	...	...	2.34	...	...
	N	3[b]	10	1 to 2	2.31	2.50	2.60	2.61	2.56	2.60	2.58	2.54	...	...	2.48	2.33	...
	P	29.82	100	0.25	2.36	2.50	2.56	2.56	2.56	2.55	...	2.53	...	...	2.46	...	...
Casein	O	24.48	40.8	5 to 6	...	...	...	...	...	1.68	...	1.68	...	...	1.66	1.62	...
Ovalbumin, D	O	32.01	53.4	3	...	...	...	1.36	...	1.40	...	...	...	...	1.41	1.44	1.42
	N	6[b]	10	<4	...	...	...	1.32	...	1.38	...	1.39	...	...	1.40	1.41	...
Conalbumin, D	O	16.03	53.4	10	...	...	...	...	...	...	...	...	...	2.91	...	2.79	2.55
	N	2[b]	10	9 to 12	...	...	...	...	...	...	...	...	2.83	...	2.93	...	...
Edestin	O	45.42	82.5	7	...	...	...	...	...	...	...	1.52	...	...	1.53	1.45	1.40
	N	4[b]	10	<4	...	...	...	1.51	...	...	...	1.53	...	...	...	...	...
Arachin	O	73.82	82.0	8 to 12	...	...	...	...	...	...	...	...	...	...	1.08	1.04	0.98
	N	6[b]	10	<4	...	...	...	1.06	...	1.08	...	1.07	...	...	...	...	...
Ox muscle	O	33.62	51.7	8	...	...	...	...	...	...	...	1.49	...	...	1.49	1.45	...
	N	4[b]	10	4 to 5	...	...	...	...	1.52	...	1.53	...	...	...	...	...	...
Zein	O	75.46	50.3	4 to 6	...	...	...	...	...	0.057	...	0.061	...	...	0.072	0.072	...
Gelatin	O	122.1	81.5	0.5 to 1	...	0.008	...	0.013	...	0.012	...	...	...	...	0.012	0.013	...
CS-54R	O	65.39	81.7	<0.25	...	0.960	0.978	0.981	...	0.988	...	0.985	...	...	0.985	0.981	0.948
CS-56R	O	122.07	81.5	1 to 1.5	...	0.540	0.541	0.549	...	0.556	...	0.561	...	...	0.561	0.561	0.540

[a] Reaction I was carried out for indicated time intervals by designated procedures. Individual optimum conditions of Reaction II were used for all proteins except zein and gelatin, for which composite optimum conditions were used.
[b] Approximate quantity used. Results are calculated from exact weight.

Table XIII. Summary of Results of Tryptophan Analyses on 13 Protein Preparations

(Precision and agreement of values obtained by various procedures)

Protein	Procedure	No. of Tests	Sample Size	Vol. of 19 *N* Acid	Optimum Time of Reaction I	Wave Length of Maximum Color, mμ	Average Tryptophan Content[a]	Average Deviation	Average Deviation	Corrected Tryptophan Content	Correction
			Mg.	*Ml.*	*Hours*		%		%	%	%
β-Lactoglobulin	N	2	3	10	3	590	2.60	±0.02	0.8	2.60	0.0
	P	3	15	50	3	590	2.58	±0.01	0.4	2.59	0.4
	O	1	60	200	6	590	2.43	...	...	2.52	3.6
	O	2	15	50	6	590	2.50	±0.01	0.4	2.56	2.3
Casein	N	4	2	10	6	610	1.66	±0.02	1.2	1.68	1.2
	N	4	4	10	6	610	1.64	±0.01	0.6	1.66	1.2
	N	4	6	10	6	610	1.65	±0.02	1.2	1.67	1.2
	O	1	24.5	40.8	6	610	1.68	...	...	1.70	1.2
Ovalbumin, D[b]	N	6	6	10	24	580	1.40	±0.01	0.7	1.42	1.4
	O	1	32	53	24	580	1.44	...	...	1.46	1.4
Ovalbumin, N(7X)[c]	K	1	6.6	10	6	580	1.41	...	...	1.43	1.4
Conalbumin, D[d]	N	5	2	10	12	620	2.93	±0.02	0.7	3.03	3.3
	O	1	16	53	10	620	2.91	...	...	3.01	3.3
Conalbumin, N[e]	K	1	3	10	4	610	3.03	...	...	3.10	2.3
Edestin	N	3	4	10	8	600	1.53	±0.02	1.3	1.58	3.2
	O	1	45	83	12	600	1.53	...	...	1.61	5.0
Arachin	N	3	6	10	8	610	1.07	±0.01	0.9	1.10	2.7
	O	1	74	82	12	600–610	1.08	...	...	1.12	3.6
Ox muscle	N	4	4	10	7	590–600	1.53	±0.01	0.7	1.54	0.7
	O	1	34	52	12	590–600	1.49	...	...	1.53	2.6
Zein	O	1	75	50	12	590	0.072	...	...	0.072	0.0
Gelatin	O	1	122	82	4	590	0.013	...	...	0.013	0.0
CS-54R	O	1	65	82	6	590–600	0.988	...	...	0.991	0.3
CS-56R	O	1	122	82	8	590	0.561	...	...	0.561	0.0

[a] Ash- and water-free basis.
[b] Heat-denatured ovalbumin.
[c] Native ovalbumin recrystallized seven times. Protein added to test in water solution.
[d] Heat denatured conalbumin.
[e] Native conalbumin; protein added to test in water solution.

Table XIV. Kinetics of Reaction I Using Derivatives of Tryptophan by Procedure O

Derivative	Time of Reaction I, Hours: 0.25	0.50	1	2	4	6	12	24	48	Optimum Time of Reaction I	Recovery: Observed	Recovery: Corrected	Correction
	% Tryptophan Recovered									*Hours*	%	%	%
N-Acetyl L-tryptophan	97.2	97.9	97.2	96.8	96.0	94.6	93.2	91.8	87.1	0.5	97.9	98.6	0.7
N-Acetyl L-tryptophan amide	91.5	92.9	94.7	95.4	95.0	94.3	93.2	91.9	89.1	2	95.4	95.8	0.4
N-Benzoyl L-tryptophan	...	...	...	101.1	101.1	98.7	97.6	97.2	94.0	2	101.1	101.1	0
Ethyl *N*-acetyl L-tryptophan	100.0	100.4	100.0	100.0	98.8	96.9	95.3	92.9	89.5	0.5	100.4	100.8	0.4
Ethyl L-tryptophan hydrochloride	92.5	93.4	95.5	95.9	95.9	96.3	96.7	95.5	94.1	12	96.7	97.9	1.2
N-Phenoxyacetyl L-tryptophan[a]	101.1	101.5	101.5	101.5	101.1	100.8	...	98.4	97.2	0.5	101.5	101.5	0
L-Tryptophan	93.7	96.2	96.6	97.0	97.4	100.1	100.5	100.5	97.8	12	100.5	100.5	0

[a] Procedure M used because of slow solubility of this solid in 19 *N* acid.

volved in setting up individual tests are eliminated, but owing to the larger sample a longer time was required to dissolve the protein than with Procedure N. Procedure P was used on β-lactoglobulin because this protein dissolved much faster in 19 *N* acid in the absence of *p*-dimethylaminobenzaldehyde and this procedure may have applications in special cases.

The rates of Reaction I by Procedures N and O were studied with eleven different proteins, and results are summarized in Table XII. In Procedure N the time required for complete solution varied from about 1 hour for β-lactoglobulin to 9 to 10 hours for conalbumin, and for the attainment of maximum color from 3 hours for β-lactoglobulin to 24 hours for denatured ovalbumin. In Procedure O the time required for complete solution varied from 15 minutes for CS-54R, a water-soluble protein, to almost 10 hours for denatured conalbumin and the time to attain maximum color varied from 6 hours for β-lactoglobulin, casein, arachin, and CS-54R to 24 hours for denatured ovalbumin and zein.

These results show that to obtain maximum color intensity in the determination of tryptophan in proteins, it is necessary to determine the optimum time for Reaction I for each protein by each method used. However, from data in Table XII a composite optimum time for Reaction I of 12 hours was chosen which gave tryptophan values with the minimum difference from those obtained when the individual optimum time of Reaction I for each protein was used. The difference between the tryptophan contents of the proteins as determined with individual and composite optimum times for Reaction I varied from a maximum of 3.7% with β-lactoglobulin to 0% with denatured conalbumin, edestin, arachin, ox muscle, zein, CS-54R, and CS-56R. The average difference for the ten proteins was 0.7%, which is regarded as typical of the error likely to occur with most proteins. Although the difference is not large, the optimum time for Reaction I should be determined if maximum accuracy is desired when analyzing new protein preparations.

The stability of the tryptophan–*p*-dimethylaminobenzaldehyde leuco compound, in the presence of the respective protein hydrolytic products, varied for different proteins and in all cases it was less than the leuco complex of free tryptophan (Table VI, *30*). Thus, in Procedure K maximum color was reached in 3 to 24 hours with different proteins and the loss in color in approximately the first 24 hours following attainment of maximum color varied from 0.7% with denatured ovalbumin to a maximum of 13% with β-lactoglobulin. In Procedure N maximum color was also obtained in 3 to 24 hours and the loss in approximately the first 24 hours following attainment of maximum color varied from 1 to 10%.

RESULTS OF TRYPTOPHAN ANALYSIS OF PROTEINS

A summary of the results of tryptophan analyses of thirteen proteins, showing the precision and agreement of values obtained by the different modifications together with the optimum times of

Reaction I and the wave lengths of maximum color, is given in Table XIII. Results are expressed as per cent tryptophan (not residue) in the ash- and water-free protein. Individual optimum conditions for Reactions I and II were used. An observed and corrected tryptophan value is given for each protein. The correction factor is based on the following considerations:

The tryptophan–*p*-dimethylaminobenzaldehyde complex from free tryptophan is stable under the conditions of Reaction I for 72 hours, as shown in Table VI (*30*), but the stability of this complex from proteins and several derivatives of tryptophan is less than this, as shown in Tables XI, XII, and XIV. Thus, maximum color was obtainable with free tryptophan, used as standard, for a time of Reaction I over twice as long as the optimum time of Reaction I for tryptophan in any protein or derivative. To compensate for these unequal rates of destruction of tryptophan in the standard and in the test substances, a correction was made by adding to the observed tryptophan value an amount equal to the difference between the maximum observed tryptophan value and the value obtained in a period twice the optimum time of Reaction I. As shown in Table XIII, the correction factor for eleven proteins, determined from results obtained by Procedure O, varied from 0% for zein, gelatin, and CS-56R to a maximum of 5% for edestin. The average value of the correction factor for all proteins was only 2.3%; where greatest accuracy is not required this correction can be neglected. The correction factor obtained when several derivatives of tryptophan were analyzed by Procedure O (Table XIV) ranged from 0 to 1.2% with an average value of 0.4%. This substantiates the validity of the correction factor applied to proteins, because the average recovery of tryptophan from the derivatives was 99.3%.

The precision obtained in the determination of tryptophan in proteins by Procedure N varied from a minimum average deviation of ±0.6% with four determinations of casein to a maximum of ±1.3% with three determinations of edestin. The weighted average deviation of 35 determinations made on β-lactoglobulin, casein, ovalbumin, conalbumin, edestin, arachin, and ox muscle by Procedure N was ± 0.88%, which is regarded as typical of the precision attainable with this method. The precision attainable with the other procedures is approximately the same as that for Procedure N.

Evaluation of the accuracy of the values obtained for the tryptophan contents of the proteins requires consideration of direct and indirect evidence. Cystine, cysteine, lanthionine, serine, and threonine destroy some tryptophan on heating in alkaline solution. But none of these amino acids destroys any tryptophan when determined by procedures similar to those used on the intact proteins; when 100 micrograms of tryptophan were determined in the presence of 10,000 micrograms of serine, threonine, or cystine the recoveries of tryptophan were 101, 100, and 100%, respectively. The tryptophan values of proteins should be higher, therefore, and more nearly accurate when determined on the intact proteins than when determined on their alkaline hydrolyzates and the values obtained by the two methods were from 13 to 32% higher when determined on the intact protein as shown in Table XV. That the differences in values obtained by the two methods are not constant is also to be expected, because the degrees of destruction caused by alkaline hydrolysis are dependent on the composition of the proteins.

Table XV. Comparison of Tryptophan Contents of Proteins as Determined on Intact Protein and Alkaline Hydrolyzate

Protein	Tryptophan Content: Hydrolyzate[a]	Tryptophan Content: Intact protein[b]	Difference
	%	%	%
Casein	1.21	1.67	28
β-Lactoglobulin	1.78	2.60	32
Ovalbumin	1.20	1.42	15
Conalbumin	2.11	3.03	30
Edestin	1.37	1.58	13
Arachin	0.85	1.10	23
Ox muscle	1.31	1.54	15
CS-54R	0.85	0.99	14

[a] Results taken from Tables III and IV. Values are averages of chemical and microbiological determinations on hydrolyzates made under conditions such that differences between chemical and microbiological values were not over 5%.
[b] Corrected tryptophan contents determined by Procedure N except for CS-54R which all determinations are by O.

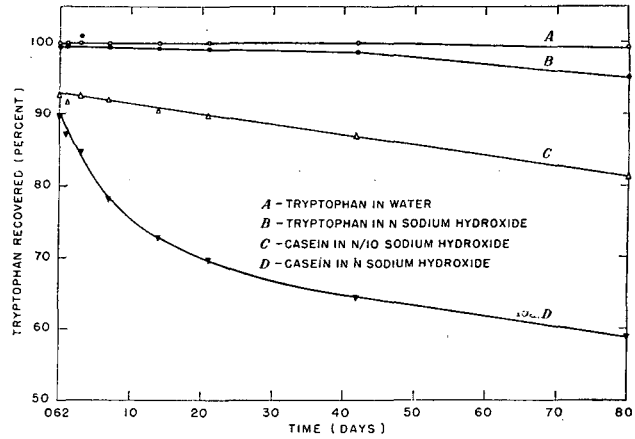


Figure 7. Comparison of Stability of Free Tryptophan in Water and Dilute Sodium Hydroxide with That of Tryptophan in Casein in Dilute Sodium Hydroxide at 25° C. in Dark

Direct evidence substantiating accuracy of the reported tryptophan contents of the proteins is the average recovery of 99.3% of the theoretical tryptophan content of six analytically pure derivatives of tryptophan (Table XIV) when analyzed by methods used for the intact proteins. Some of these derivatives contained peptide linkages and they behaved more like proteins than free tryptophan with respect to the rates of Reaction I and the stabilities of their tryptophan–*p*-dimethylaminobenzaldehyde complexes. Because of their complicated nature the determination of tryptophan in proteins is subject to more chance of error than the determination of tryptophan in relatively simple derivatives of tryptophan. However, free tryptophan was also determined in the presence of 200-fold quantities of gelatin and a tryptophan-free allergenic protein from castor beans with an accuracy of +3 and −0.2%, respectively (*30*). Therefore, protein constituents do not interfere with the tryptophan test. On the basis of these considerations the corrected tryptophan contents of the proteins given in Table XIII are believed to be accurate within ±1 to 3%, and it is anticipated that an equal degree of accuracy is attainable with other proteins, providing they do not contain unknown interfering constituents.

Discussion of the reasons for agreement or lack of agreement between tryptophan contents of proteins reported herein and those of other workers is outside the scope of this paper because of the impossibility of evaluating all factors affecting the accuracy of the various procedures.

EFFECT OF DILUTE SODIUM HYDROXIDE ON TRYPTOPHAN IN PROTEINS

Dilute sodium hydroxide is a convenient solvent for adding proteins to test solutions for tryptophan analysis. However, Tillmans and Alt (*34*) reported that the tryptophan content of casein diminished from 1.6% to about 1% on suspension in 0.1 *N* sodium hydroxide "in the cold" for 24 to 36 hours, and some destruction of tryptophan in proteins on solution in dilute sodium hydroxide would be anticipated from results reported above. To clarify this point the effect of solution and standing in 0.1 *N* and 1 *N* sodium hydroxide at 25° C. on the tryptophan content of casein was compared with the effect on free tryptophan in water

and on 0.1 *N* and 1 *N* sodium hydroxide (Table XVI and Figure 7). The tryptophan content of casein decreased from 1.56% after 1.5 hours in 0.1 *N* sodium hydroxide to 1.35% after 80 days' standing. In 1 *N* sodium hydroxide the tryptophan content decreased from 1.51% in 1.5 hours to 0.99% in 80 days. Free tryptophan, in water and in 0.1 *N* sodium hydroxide, was relatively stable, for, on standing for 80 days, recoveries of 99.2 and 98.7% of the tryptophan, respectively, were obtained while in *N* sodium hydroxide the recovery in 80 days was 95.2%.

Greater destruction of tryptophan in casein occurred on standing in 1 *N* sodium hydroxide at 25° C. for 80 days than on hydrolysis of casein with 5 *N* sodium hydroxide at 124° C. for 18 hours, as a tryptophan value of 1.2% was obtained on the hydrolyzate and 0.99% was found after standing. Greater destruction of tryptophan in casein at 25° C. in 80 days than at 124° C. for 18 hours may be because at 124° C. hydrolysis is rapid and the tryptophan exists in enol form for a short interval; at 25° hydrolysis is slow and the tryptophan would be in enol form for a longer time, during which it is more labile than when free. Furthermore, hydrolysis at 124° C. was in an inert atmosphere, whereas at 25° the solution of tryptophan was exposed to atmospheric oxygen and destruction of tryptophan in enol form may be accelerated by oxygen; an equal degree of destruction of free tryptophan in 1 *N* sodium hydroxide did not occur.

Results in Table XVI show that after solution of casein in 0.1 *N* and 1 *N* sodium hydroxide for 1.5 hours at 25° C. the tryptophan values were 1.56 and 1.51%, respectively, as compared to 1.68% when casein was not exposed to alkali. The tryptophan values of several other proteins that had been dissolved for a short time in 0.1 *N* sodium hydroxide were compared with the values obtained when the proteins were not exposed to alkali. The loss of tryptophan on solution in 0.1 *N* alkali amounted to from 7.5 to 13.4%, although denatured crystalline ovalbumin showed a gain of 3.9%; the reason for this is not known. It is apparent, however, that proteins cannot be dissolved, even briefly in 0.1 *N* sodium hydroxide, without possibility of some destruction of tryptophan and this point should be considered in the preparation of proteins and their analysis for tryptophan.

EFFECT OF CARBOHYDRATE ON THE DETERMINATION OF TRYPTOPHAN IN PROTEINS

Because many proteins or tryptophan-containing fractions obtained from natural sources contain carbohydrates, a satisfactory method of analysis should permit the determination of tryptophan in the presence of carbohydrate. It has been shown that recoveries of 98 to 100% can be obtained when 100 micrograms of free tryptophan are determined with a 50-fold quantity of glucose (*30*). Good recoveries of tryptophan were also obtained with added fructose but there was a tendency to develop interfering color in the blank and test solutions.

Results of the determination of tryptophan in casein in mixtures containing 67% glucose or fructose are shown in Table XVII. Recovery of 96% of the tryptophan was obtained when both test and blank solutions contained 5 mg. of casein and 10 mg. of glucose (test 1, blank 1); but recovery was only 85% when both test and blank solution contained 5 mg. of casein and 10 mg. of fructose (test 2, blank 4). Recoveries of 96% were obtained in both cases with the same test solutions, but with blanks that contained casein and no carbohydrate (blanks 2 and 5), and recoveries of 97 and 96% were obtained with glucose and fructose, respectively, when the blank solutions contained neither carbohydrate nor protein (blanks 3 and 6).

An interfering pink color produced with fructose and casein together in the blank solution causes an apparent loss of tryptophan, but casein alone in the blank had no appreciable effect on the recovery of tryptophan. Therefore, results accurate enough for most purposes can be obtained by omission of the carbohydrate-containing sample from the blank where an interfering color, of the type formed by fructose, occurs. The effect of fructose on the amount of sodium nitrite required for the development of maximum color was determined. It was found that with 5 mg. of both fructose and casein in the test solution and neither of them in the blank, recoveries of 96.4, 97.4, 95.8, and 94% were obtained with 0.1 ml. of 0.045, 0.060, 0.090, and 0.2% sodium nitrite, respectively. This slightly greater requirement of sodium nitrite (0.045% is optimum for casein alone) caused by the presence of fructose was neglected in the experiments shown in Table XVII, but with an unknown substance the amount of sodium nitrite required to give maximum color should be determined. The tests described in Table XVII contained more carbohydrate than will be found in most carbohydrate-protein fractions; therefore, even better than the 96 to 97% recovery of tryptophan should be obtainable. The natural proteose fractions CS-54R and CS-56R contained 6.3 and 35.2% chemically combined polysaccharidic carbohydrate, respectively, and there was no apparent interference by this carbohydrate in the tryptophan analysis of these substances.

PROCEDURES FOR DETERMINATION OF TRYPTOPHAN IN PROTEINS

General Considerations. The following procedures were designed for the determination of tryptophan in proteins and protein-carbohydrate mixtures. Before application to other tryptophan-containing mixtures it is necessary to determine whether organic or inorganic interferers are present. The colorimetric reactions of tryptophan–*p*-dimethylaminobenzaldehyde and sodium nitrite are very sensitive and good-quality or purified reagents, distilled water, and carefully cleaned glassware should be used and impure air containing such contaminants as hydrogen sulfide or oxidizing agents should be avoided. When the simple precautions given in the detailed procedures are observed, the methods give precise and accurate results.

Directions for the determination of tryptophan in proteins by both optimum conditions as determined for individual proteins and composite optimum conditions based on all the proteins studied are described. Individual optimum conditions for Reactions I and II for the proteins studied are given in Tables XII and XIII, and X, respectively. Composite optimum conditions as determined from Procedures N and O are: Reaction I, 12 hours; Reaction II, 0.1 ml. of 0.045% sodium nitrite reacting for 30 minutes. It is unfortunate that the composite optimum time of Reaction I is 12 hours; 6 or 18 to 24 hours would be more convenient. But as a few proteins require more than 6 hours for complete solution, the 18- to 24-hour period would be more generally applicable. It was calculated that the difference between tryptophan values of proteins determined from individual optimum times of Reaction I and a composite time of 24 hours varied from a maximum of 10.4% with β-lactoglobulin to 0% with ovalbumin, and the average deviation for the ten proteins was 3%. Therefore,

Table XVI. Stability of Tryptophan, Free and in Casein, in Dilute Sodium Hydroxide at 25° C.

Time of Standing	Corrected Tryptophan Content of Casein[a]		Free Tryptophan Recovered[b]		
	0.1 *N* NaOH	1 *N* NaOH	Water	0.1 *N* NaOH	1 *N* NaOH
Days	%	%	%	%	%
0.06	1.56	1.51	100	100	99.4
1	1.54	1.47	100	99.5	99.4
3	1.56	1.42	100	99.5	101
7	1.55	1.31	100	100	99.4
14	1.52	1.22	100	100	99.0
21	1.51	1.17	100	99.5	99.0
42	1.46	1.08	100	99.9	98.8
80	1.36	0.99	99.2	98.7	95.2

[a] Procedure. Samples of casein weighing 151.70 and 151.86 mg., in 50-ml. glass-stoppered, Pyrex, Erlenmeyer flasks, were dissolved in 25 ml. of 0.1 *N* and 1 *N* sodium hydroxide, respectively, by gentle agitation at room temperature. The solutions were stored at 25° C. in the dark. At desired intervals a 1.0-ml. sample of each solution was analyzed for tryptophan by Procedure L.

[b] Solutions containing 100 micrograms per ml. of free tryptophan in water and in 0.1 *N* and 1 *N* sodium hydroxide were prepared at room temperature. These solutions were stored, without preservative, at 25° C. in the dark. At desired intervals 1.0 ml. of each solution was analyzed for tryptophan by Procedure C (*30*).

Table XVII. Determination of Tryptophan in Casein in Presence of Carbohydrate

Test No.	Test Solution		Blank No.	Blank Solution			
	Carbohydrate	Casein		Carbohydrate	Casein	*p*-Dimethylaminobenzaldehyde	Tryptophan Recovered[b]
	Mg.	*Mg.*		*Mg.*	*Mg.*	*Mg.*	%
1	10.2 G[c]	5.05	1	10.1 G[c]	5.19	0	96.4
			2	0	5.02	0	95.8
			3	0	0	30	97.0
2	9.60 F[d]	5.13	4	9.98 F[d]	5.34	0	85.0
			5	0	5.02	0	96.4
			6	0	0	30	96.4

[a] Procedure N was used. Blanks containing casein and fructose became pink. When sodium nitrite was added the pink color faded somewhat but the blank solutions had a pinkish cast when transmittancies were read. Glucose had no effect on the appearance of the blank solutions containing casein.
[b] A tryptophan content of casein of 1.67% was taken as 100% recovery.
[c] Glucose.
[d] Fructose.

use of 18 to 24 hours as the composite time of Reaction I, rather than the composite optimum of 12 hours, would result in considerable convenience for the analyst with only small sacrifice in accuracy.

Reaction I was carried out at 25° ± 0.1° C., but moderate room temperature fluctuations have no appreciable effect on the results (*30*). Protection from light, especially sunlight and bright artificial light, is important during Reaction I because destruction of tryptophan in acid solution is accelerated by light (*31*). Acid solutions of *p*-dimethylaminobenzaldehyde deteriorate slightly on several days' standing (*30*) and they should not be exposed to light (*31*).

Reaction II was carried out at room temperature protected from bright light. Reaction II is an oxidative reaction; therefore, if traces of reducing agents are present the quantity of sodium nitrite to produce maximum color may be greater than required for tryptophan alone or in proteins.

Standard curves were prepared as described (*30*). The appropriate curve is indicated in each procedure.

Protein samples containing from 10 to 110 micrograms of tryptophan are required. The best range is 50 to 110 micrograms. Protein samples should be ground to pass a 60- to 100-mesh sieve to facilitate disintegration and solution in procedures where the solid is added directly to the test solution.

Blank solutions, containing a weight of protein approximately equal to that used in the test solution but no *p*-dimethylaminobenzaldehyde, were used. One tenth milliliter of the appropriate concentration of sodium nitrite was added to the blank when it was added to the test solution. Whether or not the blank contained protein had only a slight effect on the tryptophan value obtained. The apparent increase in tryptophan content when determinations were made with blank solutions containing no protein as compared to those containing protein were: casein, 1.2%; β-lactoglobulin, 1.2%; ovalbumin, 1.4%; conalbumin, 2.4%; edestin, 2.0%; and arachin, 2.5%. The average apparent increase in tryptophan content for the six proteins was 1.8%.

Dilute alkali as a solvent for proteins in tryptophan analysis should be avoided, if possible, because of destruction of part of the tryptophan. If used, it should be with consideration of this loss as demonstrated above.

A total volume of 10.1 ml. (including sodium nitrite solution) was used for individual tests which were carried out in 25- or 50-ml. Pyrex, standard-tapered, glass-stoppered Erlenmeyer flasks.

PROCEDURES K TO P

Procedure K (protein in water or alkaline solution). Eight milliliters of 23.7 *N* acid and 1.0 ml. of 2 *N* acid containing 30 mg. of *p*-dimethylaminobenzaldehyde are mixed and cooled to 25° C. To this solution is added 1.0 ml. of a water or 0.1 *N* sodium hydroxide solution containing the desired weight of protein. The test solution is mixed, cooled to 25° C., and reserved for 12 hours or for the predetermined optimum period for the protein. To the solution is then added 0.1 ml. of an 0.045% solution of sodium nitrite or 0.1 ml. of the predetermined optimum concentration for the protein. After 30 minutes or the optimum time for Reaction II, transmittancy is read and converted to weight of tryptophan from curve *C* (Figure 4, *30*), if water or 0.1 *N* sodium hydroxide is used as solvent. If more concentrated sodium hydroxide is used as solvent, a standard curve prepared similarly to curve *C* should be used, but the tryptophan should be added to the test in the same concentration of sodium hydroxide used for solution of the protein. The effect of solution of free tryptophan in water and in sodium hydroxide on the intensity of color obtained is shown in curves *B* and *D* (Figure 4, *30*).

Procedure L (protein in water or alkaline solution). When only a few tests are to be made the following modification of Procedure K may be used: To 30 mg. of solid *p*-dimethylaminobenzaldehyde are added 9.0 ml. of 21.4 *N* acid. To this solution is added the protein test solution and then Procedure K is followed.

Procedure M (protein in water or alkaline solution). For some studies it is desired to prepare several tests from a single sample, as in the determination of optimum conditions for Reaction II for an unknown protein. Sufficient solid *p*-dimethylaminobenzaldehyde, in a glass-stoppered Erlenmeyer flask, is dissolved in enough 21.4 *N* acid at 25° C., to give the desired number of 10-ml. aliquots each containing 30 mg. of the reagent. To this solution is added the protein test solution at the rate of 1 ml. for each 9 ml. of solution. The solution is mixed, cooled to 25° C., and allowed to stand for the desired time of Reaction I, after which 10-ml. aliquots are removed and the color is developed by individual or composite optimum conditions for Reaction II. Transmittancies are read and converted to weight of tryptophan as in Procedure K.

Procedure N (protein in solid form, individual tests). Solid protein, estimated to contain 50 to 110 micrograms of tryptophan, and 30 mg. of *p*-dimethylaminobenzaldehyde are weighed into a 25-ml. glass-stoppered Erlenmeyer flask to which are added 10 ml. of 19 *N* acid at 25° C. The test mixture is shaken frequently until the sample is dissolved and then allowed to stand for 12 hours or for the predetermined optimum time for Reaction I for the protein. To the solution is then added 0.1 ml. of an 0.045% solution of sodium nitrite or 0.1 ml. of the predetermined optimum concentration of sodium nitrite. After 30 minutes or the optimum time for Reaction II for the protein, transmittancies are read and converted to weight of tryptophan from curve *C* (Figure 4, *30*).

Procedure O (protein in solid form). When it is desired to prepare several tests from a single sample of protein in solid form, the following procedure is used: The desired quantity of protein for each 10-ml. aliquot is weighed into a glass-stoppered Erlenmeyer flask. Sufficient solid *p*-dimethylaminobenzaldehyde is added to give a concentration of 30 mg. per 10-ml. aliquot and 19 *N* acid, at 25° C., is added to give the desired concentration of protein. The mixture is swirled and allowed to stand with frequent shaking until all the protein dissolves. After 12 hours or the individual optimum time for Reaction I, 10-ml. aliquots are removed and color is developed by individual or composite optimum conditions. Transmittancies are converted to weight of tryptophan from curve *C* (Figure 4, *30*).

Procedure P. This procedure was used on β-lactoglobulin because this substance dissolved much more rapidly in 19 *N* acid in the absence of *p*-dimethylaminobenzaldehyde.

To 59.951 mg. of β-lactoglobulin in a 250-ml. glass-stoppered Erlenmeyer flask was added 200 ml. of 19 *N* acid. The contents of the flask, after standing a few minutes, were swirled and shaken. The protein dissolved in 15 minutes, whereas when *p*-dimethylaminobenzaldehyde was present, 3 to 4 hours were required. Twenty minutes after adding the acid, 600 mg. of *p*-dimethylaminobenzaldehyde were added and dissolved. After standing for the desired time of Reaction I, 10-ml. aliquots were removed and color was developed with 0.1 ml. of 0.045% sodium nitrite reacting for 30 minutes. Transmittancies were read and converted to weight of tryptophan from curve *C* (Figure 4, *30*). As shown in Table XII, the tryptophan value of β-lactoglobulin determined by this method agreed well with the values obtained by Procedures N and O.

METHOD FOR PROTECTION OF TRYPTOPHAN FROM CYSTINE DURING ALKALINE HYDROLYSIS

Interpretation of the effects of alkaline hydrolysis on peptide-linked tryptophan from studies using free tryptophan is difficult

Table XVIII. Effectiveness of Lead in Preventing Destruction of Free Tryptophan by Cystine on Heating in 5 *N* Sodium Hydroxide for 18 Hours[a]

Temperature, ° C.	Tryptophan Recovered[a], %: Tryptophan alone, Procedure Q[b]	Tryptophan + 2 mg. cystine Procedure Q[a]	Tryptophan + 2 mg. cystine Procedure R	Loss of Tryptophan, %: Caused by lead-cystine reaction, (col. 1 − col. 2) × 100 / col. 1	Caused by cystine, (col. 1 − col. 3) × 100 / col. 1
100	99.5 ± 0.2	90.4 ± 0.2	93.3 ± 0.3	9.2	6.1
150	97.0 ± 0.0	91.0 ± 0.0	74.3 ± 2.1[c]	6.2	23.4
185	97.0 ± 0.8	88.9 ± 0.4	59.3 ± 1.2	8.3	38.9

[a] Average of duplicate determinations.
[b] Solutions containing 1 mg. of tryptophan per ml. of lead reagent were used.
[c] Average of four determinations.

because of the unknown net effect of tryptophan-destructive and -protective reactions that might occur in the more complex proteins. A method that would eliminate or significantly modify one of these tryptophan-destructive reactions in proteins might provide substantiating evidence for the conclusions drawn from studies with free tryptophan and hence for the validity of the results of analysis of intact proteins.

A method for protecting tryptophan from destruction by cystine on heating in alkaline solution using basic lead acetate to form inert lead sulfide is described. The method was applied to certain proteins and the results confirmed conclusions based on studies with free amino acids.

PROCEDURES Q AND R

Procedure Q (use of basic lead acetate). Basic lead acetate of the type used for sugar analysis and containing 70 to 73% lead was used. Solutions were prepared fresh daily. To 1 gram of basic lead acetate in a 25-ml. glass-stoppered Erlenmeyer flask were added 10 ml. of 5 *N* sodium hydroxide. The suspension was shaken and warmed on a steam bath to about 60° C. until solution was almost complete. After cooling to room temperature the suspension was centrifuged in a capped tube and the clear solution (lead reagent) was decanted. Procedure J was used for hydrolysis, except that the lead reagent was substituted for 5 *N* sodium hydroxide. After heating, the solution was quantitatively transferred from the bomb to a 10-ml. volumetric flask and 1 ml. of 5 *N* sodium hydroxide was added during the transfer, so that the diluted hydrolyzate was 1 *N* in sodium hydroxide. The solution was centrifuged in a capped 12-ml. tube to remove lead sulfide and the clear supernatant solution was decanted.

Tryptophan was determined by an adaptation of Procedure H (*30*). To 30 mg. of *p*-dimethylaminobenzaldehyde in a 25-ml. glass-stoppered Erlenmeyer flask were added 9.0 ml. of 21.4 *N* sulfuric acid. To this solution was added 1.0 ml. of the hydrolyzate. The contents were mixed and cooled to 25° C. and allowed to stand 20 to 30 minutes in the dark at 25° C. The precipitated lead sulfate was separated by 5 minutes' centrifugation in a capped 12-ml. tube. The clear solution was decanted into a clean 25-ml. flask and reserved until the total reaction time was 1 hour. Color was developed with 0.1 ml. of 0.04% sodium nitrite solution for tests with free tryptophan and 0.07% for tests wth proteins. After 30 minutes transmittancies were determined and the solutions were treated with an additional 0.1 ml. of sodium nitrite solution. Transmittancies were determined in 10 to 15 minutes and the weight of tryptophan was calculated from the lowest transmittancy obtained using a standard curve similar to *D* (Figure 4, *30*).

Procedure R (use of basic lead acetate after hydrolysis). Heating tryptophan with sodium hydroxide and added cystine or proteins containing relatively high cystine contents produces solutions which cause an interfering color on analysis (*9*). Basic lead acetate added to such hydrolyzates precipitates the sulfur or sulfur complex so that normal color is obtained. The procedure is as follows: To the diluted hydrolyzate, in 1 *N* sodium hydroxide, is added solid basic lead acetate at the rate of 10 mg. per ml. The suspension is shaken and allowed to stand for 20 to 30 minutes, whereupon lead sulfide precipitates. The lead sulfide is separated by centrifuging and the hydrolyzate is analyzed as in Procedure Q.

RESULTS AND DISCUSSION

The effect of lead in preventing destruction of free tryptophan by cystine during alkaline hydrolysis is shown in Table XVIII. The lead reagent had no destructive effect on tryptophan alone. As shown in column 2, recoveries of tryptophan heated at 100°, 150°, and 185° C. for 18 hours by Procedure Q were 99.5, 97.0, and 97.0%, respectively, as compared to 99.3, 97.7, and 96.9%, respectively, when tryptophan was similarly heated in 5 *N* sodium hydroxide. When 1 mg. of tryptophan and 2 mg. of cystine were heated together with lead reagent by Procedure Q at 100°, 150°, and 185° C., recoveries were 90.4, 91.0, and 88.9%, respectively, but when similarly heated in 5 *N* sodium hydroxide and then treated by Procedure R recoveries were 93.1, 74.3, and 59.3, respectively, as shown in columns 3 and 4. Therefore, losses of tryptophan due to cystine were 9.2, 6.2, and 8.3% at 100°, 150°, and 185° C., respectively, when lead was present and 6.1, 23.4, and 38.9% when lead was not present (columns 5 and 6). Corresponding losses were 0, 20, and 38.1%, respectively, using microbiological analysis of hydrolyzates.

These results show that hydrolysis of proteins by Procedures Q and R might provide direct evidence for demonstrating the effects of peptide-linked cystine, serine and/or threonine on peptide-linked tryptophan. Choice of conditions and proteins for this demonstration was based on the following considerations and assumptions: (1) tryptophan values using intact proteins are accurate; (2) peptide-linked cystine, serine, and threonine destroy part of the peptide-linked tryptophan during alkaline hydrolysis of a protein, and therefore the tryptophan value obtained on the alkaline hydrolyzate of a protein containing cystine, serine, or threonine should be lower than that obtained starting with the intact protein; (3) the tryptophan value obtained on the alkaline hydrolyzate of a protein containing serine and threonine but no cystine should not be increased by use of the lead tryptophan-protective procedure; (4) the tryptophan value obtained on the alkaline hydrolyzate of a protein containing serine, threonine, and cystine should be increased by the lead tryptophan-protective procedure, but the value obtained should not equal that obtained on the intact protein; and (5) the tryptophan value obtained on the alkaline hydrolyzate of a protein containing cystine but no serine nor threonine should increase with the lead tryptophan-protective procedure and approach closely that obtained with the intact protein.

Three proteins fulfilling the cystine-serine-threonine composition requirements of conditions 3, 4, and 5 are casein, β-lactoglobulin, and subtilin, respectively. Although cystine in casein is demonstrable, the amount is low enough to be neglected for this study. Subtilin, according to Lewis *et al.* (*19*), contains no serine nor threonine, but subtilin contains 4.8% sulfur, 10% of which has been identified as lanthionine and, although unidentified, the remainder of the sulfur behaves like cystine in its tryptophan-destructive effect. In this discussion tryptophan-destructive sulfur is referred to as cystine-sulfur. β-Lactoglobulin contains all three destructive amino acids, serine, threonine, and cystine.

The effect of the lead tryptophan-protective procedure on these three proteins is shown in Table XIX and the results are summarized and correlated with their cystine-sulfur, serine, and threonine contents in Table XX. The values shown in Table XX are based on that weight of protein containing 100 micrograms of tryptophan as determined on the intact protein. The tryptophan values as determined on the alkaline hydrolyzate by Procedures Q and R are shown in columns 2 and 3, respectively, of Table XX. The cystine sulfur and the combined serine and threonine contents of the proteins are shown in columns 4 and 5. The losses of

Table XIX. Effect of Lead on Tryptophan Content of Proteins Hydrolyzed with 5 *N* Sodium Hydroxide for 18 Hours at 100° C.

Protein	Sample	Tryptophan Content[a] Hydrolyzed with Sodium Hydroxide Alone: Procedure R, lead added after hydrolysis	Hydrolyzed with Sodium Hydroxide Alone: Untreated hydrolyzate analyzed	Procedure Q (lead present during hydrolysis)	Increase in tryptophan by lead, (col. 5 − col. 3) × 100 / col. 3
	Mg.	%	%	%	%
Casein	50	1.23 ± 0.00[b]	1.23 ± 0.00	1.25 ± 0.01	1.6
β-Lactoglobulin	35	1.79 ± 0.03	1.73 ± 0.03[c]	1.94 ± 0.02	8.4
Subtilin	15	4.09 ± 0.07[d]	3.20 ± 0.02[e]	4.57 ± 0.01	11.7

[a] Average of two determinations.
[b] These results are in good agreement with those previously obtained by microbiological analysis.
[c] Analysis on hydrolyzate stored 1 day at 5° C.
[d] Average of 4 determinations. Average value obtained microbiologically on two determinations was 4.27 ± 0.11%.
[e] Interfering color caused by sulfur compounds.

Table XX. Relation of Cystine-Sulfur, Serine, and Threonine Contents of Proteins to Tryptophan Destruction during Alkaline Hydrolysis

Protein	Composition of Protein Containing 100γ of Tryptophan[a]: Tryptophan lead protection, Procedure Q	Tryptophan by alkaline hydrolysis, no lead, protection, Procedure R	Cystine cysteine-sulfur[b]	Serine + threonine[c]	Tryptophan Destroyed on Alkaline Hydrolysis: By sulfur, col. 1 − col. 2	By serine and threonine, 100 − col. 1
	γ	γ	γ	γ	γ	γ
Casein	74.4	73.2	4.8	690	1.2	25.6
β-Lactoglobulin	75.4	69.6	35.2	424	5.8	24.6
Subtilin	92.8	83.0	97.4	0	9.8	7.2

[a] Tryptophan content of casein was 1.67% and that of β-lactoglobulin was 2.57%. Tryptophan content of subtilin using intact protein by Procedure O was 4.92 ± 0.02.
[b] Calculated from data from following sources: casein, Block (*1*) and Folin and Looney (*8*); β-lactoglobulin, Brand *et al.* (*3*); subtilin, Lewis and Alderton (*19*). Sulfur of subtilin was calculated as cystine sulfur.
[c] Calculated from data in: casein, Block (*1*), Martin and Synge (*22*) and Nicolet, Shinn, and Saidel (*23*); β-lactoglobulin, Brand *et al.* (*3*); subtilin, Lewis and Alderton (*19*).

tryptophan attributed to cystine-sulfur were obtained by subtracting the tryptophan values obtained by Procedure R from those obtained by Procedure Q. These losses were 1.2, 5.8, and 9.8 micrograms for casein, β-lactoglobulin, and subtilin which contained 4.8, 35.2, and 97.4 micrograms of cystine-sulfur, respectively. These experimentally significant results show that the relative amount of tryptophan protection given by use of lead increased with the cystine-sulfur content of the protein as it should according to postulates 3, 4, and 5.

The difference between the tryptophan contents determined on the intact proteins and on the hydrolyzates made by Procedure Q was considered tryptophan destroyed by serine and threonine (column 7, Table XX). Losses of 25.6, 24.6, and 7.2 micrograms were obtained with casein, β-lactoglobulin, and subtilin which contained 690, 424, and 0 micrograms of combined serine and threonine, respectively. These significant values are in accord with postulates 3, 4, and 5. The apparent loss of 7.2% tryptophan in subtilin is attributed to the fact that protection by lead is not complete, as shown in Table XVIII, but this does not vitiate the conclusions drawn because with the other proteins the losses due to serine and threonine are several times this value.

These results confirm those obtained with free amino acids and it is concluded that peptide-linked tryptophan is partly destroyed during alkaline hydrolysis of proteins by peptide-linked cystine, serine, and threonine. That the degrees of destruction depend on the composition of the proteins is shown by the variation in difference between the tryptophan values determined on the intact proteins and their alkaline hydrolyzates (Table XV). These results show the fallacy of attempting to apply a correction factor for tryptophan destroyed during alkaline hydrolysis of a protein based on the recovery of free tryptophan added to the protein before hydrolysis.

SUMMARY

Essential features of the procedure described for studying the effects of alkaline hydrolyzing agents on free tryptophan, tryptophan and added substances, and tryptophan in proteins are: (1) use of a nickel microbomb at temperatures up to 185° C.; (2) use of an inert gas to prevent oxidation; (3) use of proteins in pellet form to prevent loss during evacuation and filling with hydrogen; (4) use of 20 to 50 mg. of protein sample; (5) yield of sufficient hydrolyzate for both chemical and microbiological analysis; (6) reproducible conditions of hydrolysis; and (7) simplicity conducive to sound quantitative technique. Recoveries of tryptophan heated with 5 *N* sodium hydroxide for 18 hours at 100°, 151°, and 185° C. by this method were 99.3 ± 0.4, 97.7 ± 0.6, and 96.9 ± 0.6%, respectively. The effects of time and temperature of hydrolysis with 5 *N* sodium hydroxide on the indicated tryptophan contents of casein, β-lactoglobulin, ovalbumin, conalbumin, edestin, ox muscle, arachin, and an allergenic polysaccharidic protein of cottonseed were determined by chemical and microbiological analysis of hydrolyzates. The effect of temperature on the rate of complete hydrolysis of casein was determined.

The effect of temperature on the rate of racemization of L-tryptophan heated in 5 *N* sodium hydroxide was determined. Complete racemization occurred in 2 and 8 hours at 185° and 151° C., respectively. The racemization occurring in various periods at 100° and 124° C. was also determined. Racemization of tryptophan in derivatives on hydrolysis with 5 *N* sodium hydroxide at 100° C. for 18 hours was determined using *N*-acetyl L-tryptophan, *N*-benzoyl L-tryptophan, *N*-phenoxyacetyl L-tryptophan, *p*-phenylphenacyl *N*-acetyl L-tryptophan, *p*-phenylphenacyl *N*-phenoxyacetyl L-tryptophan, ethyl *N*-acetyl L-tryptophan, ethyl L-tryptophan hydrochloride, and *N*-acetyl L-tryptophan amide. Results of this study are discussed in relation to Dakin's enol theory of racemization of amino acid derivatives and amino acids in proteins.

The effects on tryptophan of heating in 5 *N* sodium hydroxide at 150° C. for 18 hours individually with seventeen amino acids—alanine, phenylalanine, tyrosine, leucine, glycine, glutamic acid, proline, hydroxyproline, arginine, lysine, histidine, methionine, cystine, cysteine, lanthionine, serine, and threonine— and two carbohydrates—glucose and fructose—have been determined. Cystine, cysteine, lanthionine, serine, and threonine cause significant destruction. Cystine destroys some tryptophan in alkaline solution under tryptophan-racemizing conditions but not nonracemizing conditions. Serine destroys tryptophan under both tryptophan-racemizing and nonracemizing conditions.

The effect of nine amino acids in protecting tryptophan from destruction by serine when heated together in 5 *N* sodium hydroxide ranged from complete with hydroxyproline and histidine to practically none with proline. Amino acids in proteins also protected tryptophan from destruction by serine during alkaline hydrolysis. The relationship of structure and nature of functional groups of amino acids to both destruction of tryptophan and to protection of tryptophan from destruction by serine is discussed. A method using basic lead acetate for protection of tryptophan from cystine on heating in alkaline solution is described. The method was applied to casein, β-lactoglobulin, and

subtilin and it was concluded from the results that peptide-linked tryptophan is destroyed during alkaline hydrolysis of proteins by peptide-linked cystine, serine, and threonine and that the degree of destruction depends on the composition of the protein.

Several modifications of a procedure for the colorimetric determination of tryptophan in proteins are described. The basic method, like that for the determination of free tryptophan, involves two steps—reaction of tryptophan and *p*-dimethylaminobenzaldehyde in 19 *N* sulfuric acid to form a colorless condensation product and subsequent development of a blue color by oxidation with sodium nitrite. Unhydrolyzed proteins were used because of the destruction of some tryptophan on alkaline hydrolysis by cystine, cysteine, lanthionine, serine, and threonine and because these amino acids do not destroy tryptophan under the conditions used for analysis of the unhydrolyzed protein. For maximum accuracy proteins must be analyzed using predetermined optimum conditions for the basic reactions for each protein. A general procedure based on studies with eleven proteins may be used where resulting economy of time outweighs possible sacrifice of a small degree of accuracy. The most accurate values for the tryptophan content of the proteins obtained by averaging values obtained by the different modifications are: casein, 1.68; crystalline β-lactoglobulin, 2.57; heat-denatured crystalline ovalbumin, 1.44; native crystalline ovalbumin, 1.45; heat-denatured conalbumin, 3.02; native conalbumin, 3.10; edestin, 1.60; arachin, 1.11; ox muscle, 1.54; zein, 0.072; gelatin, 0.013; CS-54R, 0.99; and CS-56R, 0.56%. The precision of the method is ±0.88% and the accuracy is believed to be ±1 to 3%. Tryptophan may be satisfactorily determined in the presence of carbohydrates.

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Fluorescence Test for Thallium in Aqueous Solution

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Pringsheim and Vogels (*1*) report that thallous ion shows no fluorescence when excited by light of about 2500 Å., but shows bright blue fluorescence in the presence of excess chloride ion. They present quantitative measurements of the thallium fluorescence as functions of the thallium, chloride ion, and hydrogen ion concentrations. At acidities less than about 1 *N*, the intensity of the blue fluorescence increases with increasing chloride ion concentration. On the other hand, when both the hydrogen ion concentration and the chloride ion concentration are greater than about 1 *N*, a further increase in either concentration produces a decrease in the intensity of the fluorescence. Pringsheim and Vogels suggest that the $TlCl_3^{--}$ is responsible for the blue fluorescence, with neither H_2TlCl_3, formed in the presence of considerable acid, nor thallium ion showing any visible fluores-

A method for the qualitative detection of thallium, based upon the bright blue fluorescence produced in saturated sodium chloride solutions under the influence of short-wave ultraviolet light, is described. No other ions produce fluorescence that would be mistaken for that of thallium, and all known interferences are eliminated by simple procedures. Fluorescence is visible at a thallium concentration of 1 to 50,000,000 when the depth of solution observed is 4 inches. A short-wave ultraviolet lamp is the only special equipment needed.

cence. The present investigation was undertaken to develop a procedure for applying this fluorescence phenomenon to the detection of thallium in ores and other materials.

A preliminary test showed relatively little difference in sensitivity whether sodium chloride, magnesium chloride, or calcium chloride was used. The greater purity of sodium chloride far outweighs the slightly greater sensitivity obtained with the more soluble chlorides; moreover, the lower chloride concentration resulting from the use of sodium chloride allows an acidity as high as 2 or 3 *N* to be used without objectionable interference with the test from a qualitative point of view.

APPARATUS

The lamp used in this investigation was the Mineralight V-41, manufactured by Ultra-Violet Products, Inc., Los Angeles, Calif. The measured power input to the lamp was about 18 watts. Approximately 90% of the total radiation from this lamp occurs in a nearly monochromatic band at 2537 Å.

FLUORESCENCE TEST

The solution to be tested for thallium, after elimination of all interfering substances as described below, is treated with sufficient sodium bisulfite to ensure complete reduction of all thallium present, and the solution is boiled until the odor of sulfur dioxide has completely disappeared. It is important that enough acid be present to decompose all excess sulfite. The acidity should be kept as low as is convenient, although an acidity as high as 3 *N* will desensitize the test only slightly. After cooling, the solution is saturated with C.P. sodium chloride and examined under ultraviolet light in a darkroom. When the ultraviolet lamp is placed over the open top of the vessel, the presence of thallium is indicated by a bright blue fluorescence. Generally, the fluorescence will be produced more or less throughout the solution; however, if the thallium content is relatively high, the fluorescence will be limited to the upper portion of the solution because of the strong absorption of the ultraviolet light.

The solution should be examined rapidly under ultraviolet light immediately after addition of the sodium chloride, because thallium in concentrated chloride solutions is oxidized by air and the oxidation is accelerated by ultraviolet light.

It has been found generally most convenient to work with about 50 ml. of solution contained in a 250-ml. Pyrex beaker. In this case, the effect of 0.001 mg. of thallium is distinguishable from a blank when the solution is viewed horizontally. An increase in absolute sensitivity can be obtained by using smaller volumes, provided the depth of solution through which the observation is made does not become too small—for example, 0.25 microgram of thallium can be detected in a 10-ml. volume when the solution is placed in a 1.5 × 1.5 × 10 cm. glass cell.

TEST FOR INTERFERENCE

The solution is tested under ultraviolet light as described. If no fluorescence is observed, 1 or 2 drops of a thallous chloride solution containing 0.10 gram of thallium chloride per liter (one 0.03-ml. drop contains 2.5 micrograms of thallium) are added and the solution is again examined under light. If definite blue fluorescence is not produced, some interference is present which must be removed before a conclusion can be reached.

EFFECTS OF VARIOUS SUBSTANCES

To determine the possible sources of interference with this test, various metallic and nonmetallic ions were examined under the conditions described above, both alone and in the presence of thallium. These ions are grouped into three categories according to their effect.

1. The following ions in moderate quantities (ca. 0.25 gram per 50 ml.) do not interfere in any way with the thallium test as described: NH_4^+, Li^+, K^+, Ca^{++}, Sr^{++}, Ba^{++}, Zn^{++}, Mg^{++}, Be^{++}, Al^{+++}, In^{+++}, Ga^{+++}, SO_4^{--}, ClO_4^-, $C_2H_3O_2^-$, F^-, and $H_2PO_4^-$.
2. Of all the substances investigated, only six were found to show fluorescent effects under the conditions employed for the detection of thallium. Univalent mercury is precipitated when sodium chloride is added to saturate the solution, and the resulting insoluble mercurous chloride shows strong red fluorescence. The other ions, together with the colors of each fluorescence and the approximate quantity per 100 ml. of solution at which the fluorescence becomes noticeable, are: Sn^{++}, whitish green, 0.1 mg.; Pb^{++}, yellowish green, 1 mg.; Ce^{+++}, purplish white, 1 mg.; Cu_2^{++}, green, 1 mg.; and Sb^{+++}, pink, 5 to 10 mg. The fluorescence produced by these metals could not be mistaken for that produced by thallium because of the pronounced difference in the colors. Moreover, the sensitivity of the fluorescence produced by these metals is considerably less than that of thallium, and the fluorescence is produced only at the surface of the solution except when the metal concentration is at about the minimum at which the fluorescence becomes visible. However, they interfere owing to a masking effect and must be removed before the test can be applied successfully.
3. The fluorescence of thallium is interfered with in a negative way by: substances such as sulfur dioxide, iodides, and ferrous iron that quench the fluorescence; substances such as cadmium and bismuth that absorb the short-wave ultraviolet light strongly; substances such as cobalt and nickel that have absorption bands in the visible region of the spectrum and either absorb the blue light of the thallium fluorescence completely or distort it until it is no longer recognizable; and bromide ion which gives a green fluorescence possibly due to the formation of a bromide complex (*1*). The substances tested in this investigation that interfere with the production of the thallium fluorescence for any of these reasons and which must be removed are: Fe^{+++}, Co^{++}, Ni^{++}, Cr^{+++}, Cu^{++}, Cd^{++}, Bi^{+++}, Hg^{++}, Sn^{++++}, Fe^{++}, Au^{+++}, Pt^{++++}, Ti^{++++}, AsO_3^{---}, SO_3^{--}, NO_3^-, NO_2^-, S^{--}, I^-, Br^-, SCN^-, VO_3^-, MoO_4^{--}, and H_2O_2.

SEPARATION OF THALLIUM

A method of separation that was investigated is based on the removal of heavy metals by precipitation with sodium carbonate after reduction of the thallium by sulfur dioxide. A little sodium hydroxide is also used to make the separation of ferrous iron and cuprous copper more complete. This single separation is not completely satisfactory, however. It does not remove certain substances, notably arsenic and antimony, which interfere with the fluorescence test; and thallous ion in alkaline solution is oxidized by oxygen of the air with resulting precipitation of the extremely insoluble thallic hydroxide. This loss of thallium in the carbonate precipitate can become almost complete with small quantities of thallium, especially if the solution is boiled or allowed to stand very long before being filtered. However, if the precipitation is made at room temperature and the solution filtered as rapidly as possible, the loss of thallium is relatively unimportant for qualitative purposes.

The procedure used to separate thallium in the present qualitative test is extraction by dithizone (*2*).

After evaporation of the dithizone extract and oxidation of organic matter, any thallium present is obtained in 1 ml., or slightly less, of concentrated sulfuric acid. Twenty-five milliliters of water are added, followed by sufficient sodium bisulfite to produce a distinct odor, and the solution is boiled until the odor of sulfur dioxide has disappeared. After thorough cooling, the solution is filtered through a small filter paper containing a little paper pulp

to remove the small quantity of lead sulfate that will generally be present. Washing may be omitted. If this filtration is neglected a light greenish fluorescence may be observed in the subsequent fluorescence test if the quantity of thallium present is small. The solution is then diluted to 50 to 100 ml. and divided into two parts, one of which is saturated with sodium chloride and tested for fluorescence. If no fluorescence is observed and the test for interference indicates the presence of interfering substances (probably bismuth or cadmium), the second part is treated with a small quantity of sodium bisulfite and then with sufficient solid sodium carbonate to make it slightly alkaline. The solution is immediately poured through a moderately fast filter paper. As the precipitate formed is small and little time is required for filtration, the loss of thallium by oxidation or occlusion is negligible. The filtrate is acidified with enough sulfuric acid to react completely with all carbonate and sulfite present and leave the solution slightly acidic. After carbon dioxide and sulfur dioxide are removed by boiling, the solution is cooled, saturated with sodium chloride, and tested for fluorescence. Although this separation could be applied to the total solution after the filtration of lead sulfate, it cannot be combined with this filtration owing to the solubility of the lead sulfate in alkaline solutions; it will generally save time to test half the solution to find out whether the second separation is necessary.

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Iodometric Semimicrodetermination of Thallium in Ores and Flue Dusts

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An accurate semimicromethod for the determination of thallium is described. Sample decomposition is effected by pyrosulfate fusion, followed by separation of thallium from essentially all interferences by extraction from ammoniacal citrate-cyanide solution with a chloroform solution of dithizone. The isolated thallium is then determined by an iodometric procedure. The method is applicable to material containing a few thousandths per cent or more of thallium.

AMONG the most commonly used methods for determining small quantities of thallium are those based on colorimetric determination of iodine. In the method of Haddock (*3*), thallium is extracted from an ammoniacal citrate-cyanide solution by a chloroform solution of dithizone; the thallium is then determined colorimetrically from the blue color produced by the action of trivalent thallium on excess iodide in the presence of starch. In Shaw's method (*8*), thallium is separated by ether extraction; the iodine liberated by the action of trivalent thallium on iodide is dissolved in carbon disulfide and determined colorimetrically.

Titration of the iodine would have distinct advantages as to accuracy, speed, and ease of application to large numbers of samples simultaneously. The work of Shaw and Haddock on the completeness of iodine liberation seemed to assure the success of the method, but preliminary experiments showed that, although the iodine liberated could be titrated with thiosulfate using starch as indicator, the results were highly empirical. An iodometric determination of large quantities of thallium, using chloroform and potentiometric means to indicate the end point, was employed successfully by Hollens and Spencer (*4*), but their method fails with small quantities of thallium. Čůta (*2*) and Proszt (*7*) have described iodometric methods using starch as indicator, but Čůta's method deals with large quantities of thallium, while Proszt's, although applicable to relatively small quantities of thallium, is highly empirical. In the latter, it is necessary that the thiosulfate solution be standardized not only against thallium but against almost the same quantity of thallium that will be found in the unknown sample. The method is less sensitive than would be desirable. The present investigation of the factors affecting the thallium-iodide reaction has resulted in a method that is more sensitive and of greater range than that of Proszt, and is essentially stoichiometric.

PROCEDURE

Preparation of Solutions. SODIUM CITRATE. Add 200 grams of citric acid and several drops of thymol blue indicator to 250 ml. of water contained in a 1-liter beaker. Place the beaker in a trough of cold water, and add concentrated sodium hydroxide solution slowly and with vigorous stirring to prevent the solution from becoming unduly hot. After the citric acid has dissolved, cool, and continue the neutralization until the indicator color changes to its blue alkaline form. Dilute to 600 ml.

SODIUM CYANIDE (sulfide-free). Dissolve 200 grams of sodium cyanide in 400 ml. of water. The resulting solution will have a volume of 500 ml., 5 ml. of which will contain 2 grams of sodium cyanide. To this solution add a dilute solution of lead acetate (1.83 grams of lead acetate trihydrate per 100 ml.) until the brown color stops getting darker. Keep the excess of lead as small as possible. Warm the solution with stirring until the precipitate of lead sulfide flocculates, and filter through a rapid filter. Test the first few milliliters of the filtrate with a few more drops of the lead solution to be sure that all sulfide is precipitated. This solution may be prepared in large batches if stored in a waxed, brown-glass bottle.

HYDROXYLAMINE.* Add 20 grams of hydroxylamine sulfate and a few drops of thymol blue indicator to 50 ml. of water. Add concentrated sodium hydroxide solution with cooling until the salt has dissolved, and neutralize the solution to the indicator end point. Dilute the solution to 100 ml.

DITHIZONE. Dissolve 1.5 grams of dithizone in 1 liter of chloroform.

WASH SOLUTION.* Dissolve 5 grams of secondary ammonium citrate, $(NH_4)_2HC_6H_5O_7$, or an equivalent amount of citric acid, in 1 liter of water, and neutralize to the blue alkaline color of thymol blue indicator with concentrated ammonia. Add about 1 ml. excess of ammonia and 25 ml. of sodium cyanide solution, pH = 9.7 to 10.0.

* All starred solutions should be prepared when ready to use. The others may be prepared in larger batches if the volume of work warrants it.

BROMINE SOLUTION.* Dissolve 100 grams of sodium dihydrogen phosphate monohydrate and 100 grams of ammonium chloride in 900 ml. of freshly saturated bromine water.

PHENOL. Dissolve 25 grams of phenol in 100 ml. of glacial acetic acid. This solution is conveniently dispensed from an all-glass dropping bottle.

POTASSIUM IODIDE.* Dissolve 0.5 gram of potassium iodide in 100 ml. of water.

STARCH. Make 1 gram of Bakers C.P. soluble starch, "according to Lintner," into a paste with 5 ml. of water, and add the suspension slowly with continuous stirring to 40 ml. of boiling water. Add 50 ml. of pure colorless glycerol, and boil in an uncovered beaker until the volume has been reduced by about 25%. This should require 15 to 20 minutes. If boiling is prolonged and the volume is reduced by 40 to 50%, the solution will become turbid on cooling and standing. This turbidity can, however, be cleared by adding 5 to 10% water and warming. If the temperature is allowed to go above about 115° C., as will happen if most of the water is expelled, the starch solution will be useless. It is advisable to mark the beaker at proper reference points. Store in an amber-colored bottle.

THIOSULFATE.* Dissolve 250 mg. of sodium thiosulfate pentahydrate in a little water, and dilute to 1 liter. If the salt at hand has lost considerable water of hydration, use sufficiently less than that indicated—e.g., 200 mg.—to give a 0.0010 *N* solution. Standardize against 2 mg. of thallium under the conditions outlined below for the titration.

THALLIUM. Prepare a stock solution by dissolving 0.9880 gram of thallium sulfate or 0.9388 gram of thallium chloride in the required quantity of water and diluting to 1 liter. As required, dilute 100 ml. of this solution to 1 liter. Twenty-five milliliters of the latter solution contain 2.000 mg. of thallium.

Decomposition of Sample. Weigh a 1-gram sample of ore or flue dust into a 250-ml. Erlenmeyer flask, and add 10 grams of sodium sulfate and 5 ml. of concentrated sulfuric acid. Place the mixture on a hot plate, or heat carefully over a blast lamp, until the evolution of sulfur (from pyrites), selenium, etc., is nearly complete. Fuse the mixture over a blast lamp until a clear melt is obtained. The addition of a little solid sodium fluoride to the molten mixture after most of the sulfuric acid has been driven off is of help with siliceous material. Treat the cooled melt with about 75 ml. of water and a small pinch of sodium bisulfite, enough to produce a strong odor of sulfur dioxide. If the melt was heated long enough so that it tended to solidify or crust over, add 1 or 2 ml. of concentrated sulfuric acid also. Boil until all soluble salts have dissolved, cool thoroughly, and filter through a retentive paper into a 400-ml. beaker, using paper pulp to secure a clear filtrate. Wash with cold 2% sulfuric acid solution. The insoluble material will often be gray or black, or sometimes reddish, owing to reduction of mercury, selenium, etc., to the metallic state by sulfur dioxide.

Separation of Thallium. To the filtrate, add 5 ml. of sodium citrate solution and 5 or 6 drops of 0.04% thymol blue indicator Neutralize, with sodium hydroxide solution, as much of the free acid in the solution as possible without getting a permanent precipitate of any kind. If the indicator color can be seen, stop the addition of sodium hydroxide when the red color begins to turn to the yellow or neutral form. Complete the neutralization with concentrated ammonium hydroxide, adding 2 or 3 ml. in excess. If, however, insufficient acid is present (after partial neutralization with sodium hydroxide) to neutralize 3 to 5 ml. of ammonium hydroxide, add enough sulfuric acid to take care of this amount of ammonia, and again neutralize the solution with ammonium hydroxide. The neutral point is clearly indicated either by a change in the indicator color to blue, if the solution is relatively free of heavy metals, or by the production of strong colors in their presence.

Add 15 ml. of the cyanide solution and 5 ml. of the hydroxylamine solution, and let stand for a few minutes. If the color of the solution fades to the clear blue of the indicator, or nearly so, transfer the solution to a 250-ml. separatory funnel, dilute to 150 to 200 ml., and extract with dithizone. If a deep red color is produced which does not show signs of fading on a few minutes' standing, indicating much iron, heat the solution to about 90° C. (do not boil), let stand for about 5 minutes, and then set the beaker in a tray of running water (in a hood). When the solution has thoroughly cooled, transfer it to the separatory funnel and extract with dithizone.

Add 10 ml. of the dithizone solution to the solution in the separatory funnel, and shake vigorously for 20 to 30 seconds. Allow a few minutes for the layers to separate, then draw off the chloroform layer into a 125-ml. Erlenmeyer flask without allowing any of the froth to get into the stopcock. Repeat the extraction with successive 10-ml. portions of dithizone solution until the extract remains the brilliant green of unchanged dithizone. Make at least one additional extraction after the first bright green one that is obtained, and combine all extracts. If a very complete separation of layers is not possible, make enough additional extractions to recover completely by dilution the first extract that remained unchanged. Generally, three extractions should be sufficient, as the second extract usually will remain unchanged. If it does not, it is generally indicative of extraction of zinc, cadmium, etc., owing to insufficient cyanide. In this event, add more cyanide solution, and shake the solution again before drawing off the extract. The analyst should keep in mind the possibility of actually having enough thallium present to give more than one red extract, but this will occur infrequently—e.g., with thallium concentrates.

After extraction, discard the aqueous layer, and rinse the separatory funnel thoroughly with water. Pour the combined chloroform extracts into the separatory funnel, and wash the flask with 50 ml. of wash solution followed by two small rinses with distilled water, adding all washes to the separatory funnel. Shake the extract vigorously for 30 seconds; and after allowing the layers to separate, draw off the chloroform layer into the same 125-ml. Erlenmeyer flask. Extract the aqueous wash layer with one or two 5-ml. portions of dithizone to recover any traces of thallium that may have gotten into the aqueous layer and to make the recovery of the main extract complete. Add this extract to the main one in the flask.

To the chloroform extract containing the thallium, add 1 ml. of concentrated sulfuric acid, and evaporate the chloroform gently on a hot plate. As soon as the chloroform has evaporated but before the organic matter begins to char, add 1 ml. of 72% perchloric acid, and heat gently until all water has been expelled and the reaction proceeds quietly. If the temperature is too high, the sample tends to spatter from the water, condensing on the walls of the flask and running back into the hot sulfuric acid. This spattering, of course, can lead to mechanical losses of thallium, which may become serious considering the very small volume of solution and is especially annoying if 40 or 50 samples are being run at the same time. By having a considerable excess of perchloric acid present and allowing the decomposition to take place rather slowly at a fairly low temperature, spattering is avoided almost completely and personal attention is unnecessary. No explosions or even vigorous reactions have ever been noticed under the conditions described for oxidation of this particular type of organic matter. However, the potentialities of mixtures of organic matter and perchloric acid should be kept in mind, and extreme caution exercised.

After 10 to 15 minutes, the solution will be a light, clear red or yellow. At this point, place the solution on the hottest part of the hot plate, and evaporate to fumes of sulfuric acid. When the sulfuric acid has become colorless, cool the flask, and rinse the sides as well as the top thoroughly with several drops of water. Add a few drops of perchloric acid, and evaporate the solution again to fumes of sulfuric acid. The temperature should be high enough to decompose the perchloric acid, as indicated by the formation of a yellow color which disappears in a few minutes. When the perchloric acid has been completely volatilized, the solution should be absolutely colorless, and the total volume (concentrated sulfuric acid) will be slightly below 1 ml. Add 25 ml. of water, and complete the determination as described below. The presence of small amounts of organic matter left in the solution has been known to produce a return of the blue starch-iodine color. With appreciable amounts of organic matter, return of the blue color may be so fast as to obliterate the end point completely. By repeating the evaporation to fumes with a little perchloric acid after rinsing, organic matter is satisfactorily eliminated.

Determination of Thallium. To the thallium solution obtained above, or to a 25-ml. aliquot of the standard thallium solution to which has been added 1 ml. of concentrated sulfuric acid, add 25 ml. of the bromine reagent. Add a boiling chip to ensure smooth evolution of bromine and to prevent bumping, and heat the solution to boiling. When the bright red solution has faded to a light yellow (which will require less than 3 minutes' boiling), cool the flask rapidly by placing it in a trough of cold, running water. The bromine must not be removed entirely by boiling, for there is danger of reducing some thallium in the hot solution after all bromine has been removed. After cooling the solution to the temperature of the cold water bath, remove the bromine still remaining by the addition of 0.2 ml. (4 or 5 drops) of the phenol solution. Add 5 ml. of the potassium iodide solution; after swirling for a few seconds to ensure complete reduction of the thallium, add 1 ml. of the starch solution. Titrate the liberated iodine with 0.001 *N* sodium thiosulfate solution until the blue color is discharged.

The rate at which the thiosulfate is added throughout the main part of the titration has very little effect; the only error noted was a slight loss of iodine when the entire titration was carried out by adding a single drop at a time with constant swirling. How-

ever, the speed of titration becomes considerably more important near the end point. The color changes are not so rapid as desirable, and time must be allowed for the system to reach equilibrium. After addition of each drop near the end point, the blue color will fade rapidly (but not instantaneously), followed by a slight deepening of the color on standing for a few seconds. This deepening of the color decreases with each drop until the end point drop produces a sharp change to colorless, which will not return for several minutes. The error introduced by not allowing enough time at the end point is small and should never amount to more than 2 or 3 drops (high or low), but is important if precision results are to be obtained.

Exposure of the solution to sunlight or even skylight will result in a titer 2 or 3 drops high. The sharpness of the end point is affected somewhat by the spectral quality of the light, the end point being more easily visible when viewed against the white porcelain base of the titrating stand in diffuse daylight than under artificial lights or in brilliant daylight. It is advisable to pull the blinds on all windows facing the sun and conduct the titration in diffused daylight with such supplemental lighting from artificial lights as may be required. If the fading produced by each drop is observed, and a comparison solution of about the same volume of distilled water is used, the end point can be detected to 1 drop, or even to 0.5 drop with a little experience. The end point is not stable indefinitely but is stable enough to ensure a very sharp break at the end point, even allowing about a minute for the endpoint drop.

DEVELOPMENT OF PROCEDURE

In the development of the procedure, known quantities of thallium were titrated with an independently standardized thiosulfate solution under various conditions. From the data obtained, the optimum conditions for accurate determination of thallium were established. This procedure for determining thallium was then used to study the separation of thallium and to establish possible errors introduced in the decomposition of the sample. Each of these three main divisions of the method is discussed below in the order in which it was worked out. Calibrated weights and volumetric apparatus were used throughout the work, and all data shown are the corrected values.

The thallium solutions were prepared as described under Preparation of Solutions, using both thallous sulfate and thallous chloride to give standard solutions from two sources. Each salt was recrystallized from water which had been slightly acidified with the corresponding acid; the products were dried at 400° and 200° C., respectively. In most of the work a 25-ml. pipet (24.96 ml.) was used to deliver 1.996 mg. of thallium from either solution.

The normality of the thiosulfate solution used in the thallium titration was determined by an indirect method rather than by direct standardization of such a weak solution. An approximately 0.05 *N* solution, prepared and stabilized according to the directions of Kassner and Kassner (*5*), was standardized frequently against potassium iodate (*6*) and potassium dichromate (*9*). A 20-ml. aliquot of this standardized solution was then diluted to 1 liter to obtain a working solution of approximately 0.001 normality, the exact normality of which was obtained by calculation from the stronger standardized one.

In all work in which thallium solutions were titrated under variable conditions, duplicate thallium titrations were carried out at the same time under the standard conditions. This provided an empirical standardization as well as a theoretical one. Generally, the titration of the thallium standard agreed with the calculated value to less than 1 part per thousand, but occasionally they differed by 1 or 2 parts per thousand. This difference is due to small errors arising from different light conditions, starch indicator, or a possible difference in the actual normality of the thiosulfate solution from that calculated by dilution, all of which may vary slightly from day to day. As these errors are small and reproducible under any given set of conditions, they can essentially be canceled by an empirical standardization. Accordingly, independent standardization of the thiosulfate is recommended only when pure thallium salts are not available.

Determination of Thallium. Stoichiometry of Thallium-Iodide Reaction. Aliquots of each thallium solution (25 ml., 1.996 mg. of thallium) were titrated with standard thiosulfate solution according to the standard procedure given above. The results listed in Table I show that the iodometric determination of thallium is accurate to somewhat less than 1 part per thousand, at least in the determination of 2 mg. To determine whether the stoichiometric relationship holds for other quantities of thallium, different quantities of thallium were titrated (Table II). The data show that the relationship is satisfactorily linear up to about 3 mg. of thallium, above which the results tend to be slightly low. The last column shows the error found in another series that was titrated in a brightly lighted room but not in direct sunlight, all other conditions being the same.

Effect of Iodide Concentration. One of the most important single conditions governing the success of the titration is the quantity of potassium iodide used. If insufficient iodide is present, low results are obtained. On the other hand, if enough iodide is added to cause precipitation of a small quantity of thallous iodide, the results are also low; if relatively large quantities of thallous iodide are precipitated, no end point is visible through the muddy greenish-brown solution. It is thought that the thallous iodide adsorbs iodine, which then reacts incompletely until a considerable excess of thiosulfate has been added.

The optimum quantity of potassium iodide finally selected was the 25 mg. used in the 50-ml. volume as described in the method. This concentration gives results that are satisfactorily linear up to about 3 mg. of thallium and are less than 0.5% in error up to 5 mg. Because 5 mg. of thallium would require a 50-ml. titration, this range is satisfactory.

Effect of Other Metals. To determine the effect of other metals on the thallium titration a series of standards was titrated in the presence of various metals which are generally encountered in flue dusts, as well as certain metals which it was thought might possibly interfere when present in trace amounts. The titration of 1.996 mg. of thallium in the presence of 25 mg. of lead, bismuth, cadmium, zinc, iron, or manganese was 19.32 ± 0.02 ml. compared with the calculated value of 19.32 ml. of 0.001011 *N* thiosulfate. Titrations of 19.33, 19.42, and 19.39 ml. were obtained in the presence of 5 mg. of arsenic, antimony, and copper, respectively. Although the results with antimony and copper are slightly high, the quantity of either metal likely to be carried

Table I. Titration of Standard Thallium Solution with Standard Thiosulfate

Tl Taken, Mg.			Tl Calcd., Mg., Using Normality from	
Tl_2SO_4	TlCl	Titration, Ml.	Iodate (0.001012)[a]	Dichromate (0.001012)[a]
1.996	...	19.29 ± 0.01[4]	1.995	1.995
...	1.996	19.30 ± 0.01[4]	1.996	1.996
			(0.001011)[a]	
1.996	...	19.31 ± 0.02[3]	1.995	...
...	1.996	19.32 ± 0.02[3]	1.996	...

[a] Subscripts indicate number of runs in averages.

Table II. Effect of Quantity of Thallium on Thiosulfate Titration

Tl Taken	Titration	Calcd. Vol. (0.001011 *N*)	Error	Error in Light
Mg.	*Ml.*	*Ml.*	*Ml.*	*Ml.*
0.040	0.40	0.39	+0.01	+0.03
0.200	1.95	1.94	+0.01	+0.06
0.400	3.90	3.87	+0.03	+0.05
0.801	7.75	7.75	0.00	+0.05
1.200	11.63	11.61	+0.02	+0.10
2.001	19.39	19.37	+0.02	+0.13
2.802	27.10	27.12	−0.02	+0.14
3.602	34.80	34.86	−0.06	+0.11
4.403	42.46	42.62	−0.16	+0.03
5.203	50.20	50.36	−0.16	+0.02

Table III. Other Factors

	Variations from Standard Procedure	Titration[a], Ml.
1.	5 ml. H_2SO_4 present	19.40
2.	0 ml. H_2SO_4 present	19.25
3.	Fast addition of titrant	
	18.8 ml. of titration conducted with buret stopcock fully open, solution swirled continuously	19.32
4.	Slow addition of titrant	
	Titration conducted by adding single drop at a time	
	Slow continuous swirling of solution at temperature of cooling bath (15° C.)	19.32
	Vigorous agitation of solution at temperature of cooling bath	19.28
	Slow continuous swirling of solution at room temperature (28° C.)	19.24
5.	Solution allowed to stand 1 hour after elimination of bromine by phenol	19.32
6.	Solution allowed to stand quietly for 30 minutes after liberation of iodine by addition of potassium iodide	
	In cooling tray	19.31
	At room temperature	19.08

[a] Calcd., 19.32 ml.

through the extraction is much less than 5 mg., even if the original sample consisted of the pure metal. Hence, there is little likelihood that these metals will interfere with the determination.

The presence of 25 mg. of mercury caused low results (18.6 ml.), owing, probably, to a reduction of the iodide concentration through formation of the complex HgI_4^{--}. The method, however, amply provides for removal of this element.

Titration Blank. Solutions carried through the titration without any thallium present fail to produce a blue color on the addition of iodide and starch but produce a definite blue color on the addition of a single drop of 0.001 *N* iodine solution. The absence of a reagent blank is understandable, for the method is very insensitive toward traces of iron, copper, arsenic, etc., that may be present in the reagents used. However, the absence of an indicator blank is remarkable, considering the thiosulfate concentration employed, and is attributed to the very sensitive nature of the starch-glycerol reagent under the conditions of volume and concentration of iodide and neutral salts in the present method. However, there are two sources of error that will not be detected unless a titration is actually obtained in the blank determination. The first is due to the accelerating action of ultraviolet light on the air oxidation of iodide, which leads to slightly high results as indicated in column 5 of Table II. The second is concerned with the discharge of the blue starch-iodine color; if the starch solution is more than 2 or 3 days old, the end point is less sharp and 2 or 3 drops excess of thiosulfate are required before the blue color is discharged. If an error of this magnitude can be tolerated, the starch-glycerol reagent can be used for at least 6 months. Inasmuch as both errors depend somewhat on the quantity of thallium titrated, no correction is feasible. A small quantity of thallium (about 0.05 mg.) should be included in the blank as a check on laboratory conditions rather than to obtain a corrective blank in the usual sense. By taking adequate precautions against ultraviolet light and using freshly prepared starch-glycerol reagent, it has been consistently possible throughout this investigation to obtain results with no significant blank.

Other Factors. The data listed in Table III indicate that normal errors in estimating the 1 ml. of sulfuric acid required in the method will not result in appreciable error; that speed of titration does not affect the titration directly; and that loss of iodine will generally be negligible if the titration is carried out rapidly without allowing the solution to warm up or to be agitated vigorously.

Separation of Thallium. The extraction of thallium by a chloroform solution of dithizone, although using more expensive reagents than other methods, is a very satisfactory method for separating thallium, because it is rapid and complete and few substances interfere. From an ammoniacal citrate-cyanide solution, only lead, stannous tin, and bismuth are coextracted with the thallium. After an oxidative attack and filtration of a sulfate solution, only bismuth, which is not likely to be encountered in great quantity, and small amounts of lead, owing to the solubility of lead sulfate, will be extracted, inasmuch as stannic tin is not reduced by hydroxylamine (*1*). In the present method, neither lead nor bismuth in moderate quantity interferes in the subsequent iodometric determination of thallium, and hence both may be ignored. However, large quantities of bismuth will require a prohibitive number of extractions for complete removal in the separation and will also interfere in the subsequent titration, owing to a masking effect of the BiI_4^- color on the end point. Such large quantities, when encountered, should be removed by an appropriate separation.

The brilliant green color of unchanged dithizone is generally used as an indicator of the end of extraction. However, a seemingly endless series of blue-green to red extracts may sometimes be obtained after all the thallium has been extracted. For example, certain metals, especially cadmium, may be extracted in sufficient quantity to distort the color of the dithizone extract unless the cyanide content is several times that required to form the complex. In extreme cases, as in the determination of thallium in cadmium metal, the cyanide concentration required to prevent extraction of cadmium may approach 10 to 20%. Such high cyanide concentrations will not affect the extraction of thallium in any way, provided the cyanide is free of sulfide. The small quantity of sulfide present in C.P. sodium cyanide is sufficient to cause serious error if not removed.

Another cause is oxidation of dithizone by ferric iron, which is easily recognized by the characteristic formation of a creepy, red precipitate on the walls of the funnel above the aqueous solution and a green sheen around the extract. The ferric citrate complex is not reduced by hydroxylamine in alkaline solution and the ferricyanide complex, although easily reducible, is formed very slowly at room temperature. If the solution is heated and allowed to stand hot for a few minutes, the formation of ferricyanide (and subsequent reduction) is so complete that even large quantities of iron will cause no interference. However, because alkaline ammonium cyanide solutions are decomposed on boiling, with formation of a deep red color and a dark, voluminous precipitate, the solution should be heated as little as possible consistent with the quantity of iron to be complexed.

A third cause of extended extractions is the distribution of dithizone at high pH. Above a pH of about 10.2, the distribution of dithizone is so heavily in favor of the alkaline aqueous layer that the color of the chloroform layer is strongly affected by the light red color of the small quantities of alkali-insoluble impurities present in the dithizone. As shown later, however, high extraction efficiency is obtained at high pH values, at least up to 11.8, so that the end of the extraction can be determined by the weakness of the extract color rather than by the brilliant green color of excess dithizone. One additional extraction should then suffice for complete extraction.

To determine the effect of pH on the extraction of thallium by dithizone, the pH was regulated in a manner similar to that used in the regular method by neutralizing various quantities of sulfuric acid with concentrated ammonium hydroxide and adding a constant excess of 1 ml. of the ammonium hydroxide together with various quantities of cyanide. A rather large volume of aqueous solution (200 ml.) was used to accommodate better the salts from large samples, fusions, etc., and to make washings on a routine basis more convenient than with smaller volumes, even though the latter would result in greater efficiency. The extraction was carried out by shaking the solution vigorously for 30 seconds with a 10-ml. portion of dithizone, drawing off the extract as completely as possible, and then allowing 10 ml. of chloroform to drop through the solution without shaking to wash out the stem of the funnel. This extraction and washing process was repeated four times and the thallium content of each extract determined by titration. The results are listed in Table IV.

From these data it is concluded that maximum extraction

Table IV. Effect of pH on Extraction of Thallium

Test No.	H_2SO_4	NaCN	pH	Titration of Extracts 1	2	3	4	Total[a]
	Ml.	*Grams*		*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
1	10	1	8.7	16.09(BG)[b]	2.52(Dz)[b]	0.55(Dz)[b]	0.13(Dz)[b]	19.29
2	10	5	9.05	17.37(BG)	1.78(Dz)	0.22(Dz)	0.00(Dz)	19.37
3	10	10	9.2	17.70(PG)	1.50(Dz)	0.16(Dz)	0.00(Dz)	19.36
4	10	20	9.35	18.15(P)	1.12(Dz)	0.10(Dz)	0.00(Dz)	19.37
5	2	1	9.2	17.70(PG)	1.49(Dz)	0.17(Dz)	0.00(Dz)	19.36
6	2	5	9.5	18.30(P)	1.01(Dz)	0.05(Dz)	0.00(Dz)	19.36
7	2	10	9.7	18.40(P)	0.90(Dz)	0.00(Dz)	0.00(Dz)	19.30
8	2	20	9.85	18.50(P)	0.82(Dz)	0.00(Dz)	0.00(Dz)	19.32
9	0	1	11.15	18.17(R)	1.10(YB)	0.10(YG)[c]	0.00(YG)[c]	19.37
10	0	5	11.5	18.55(R)	0.80(YB)	0.00(YG)[c]	0.00(YG)[c]	19.35
11	0	10	11.65	18.44(R)	0.92(R)	0.00(R)[c]	0.00(R)[c]	19.36
12	0	20	11.8	18.43(R)	0.86(R)	0.00(R)[c]	0.00(R)[c]	19.29

[a] Calcd., 19.32 ml.; standard, 19.35 ml.
[b] Letters in parentheses refer to extract color: BG, bluish green; PG, purplish green; YG, yellowish green; YB, yellowish brown; P, purple; R, red; Dz, brilliant green of dithizone.
[c] Relatively weak colors.

efficiency is obtained at a pH greater than about 9.5; and the mechanics of the extraction are best at a pH below about 10.2, where the chloroform still contains enough dithizone to act as an indicator of the end of the extraction. This range is easily and consistently obtained simply by neutralizing 1 to 2 ml. of concentrated sulfuric acid with concentrated ammonium hydroxide (3 to 5 ml.), adding 1- or 2-ml. excess, and using at least 5 grams of sodium cyanide.

Table V. Effect of Acids on Loss of Thallium

	Treatment of Thallium	Titration[a], Ml.
1.	25 ml. concd. H_2SO_4 boiled off	19.31
2.	Tl evaporated with H_2SO_4 and Tl_2SO_4 allowed to stand on hot plate (ca. 350° C.) for 2 hours	18.8
3.	Sample of Tl_2SO_4 heated at 400° C. in electric furnace for 2 hours	17.7
4.	Three evaporations with 25 ml. concd. HCl after reduction of Tl with SO_2	19.33
5.	Three evaporations with 20 ml. HCl and 5 ml. HNO_3	
	To 5-ml. volume	19.1
	To dryness	14.5
6.	Three evaporations with 25 ml. concd. HNO_3	
	To 5 ml. volume	19.32
	To dryness	18.6
7.	Three evaporations to fumes with 20 ml. HCl and 5 ml. HNO_3 in presence of 5 ml. H_2SO_4	19.31
8.	Two evaporations to fumes with 25 ml. HNO_3 in presence of 5 ml. H_2SO_4	19.33
9.	5 ml. concd. HNO_3 added to 5 ml. concd. H_2SO_4 as latter was just beginning to fume. Repeated 5 times	19.27
10.	Two evaporations with 10 ml. 72% $HClO_4$ in presence of 1 ml. H_2SO_4	19.33
11.	Pyrosulfate fusion[b]	19.33
12.	Pyrosulfate fusion plus three 0.5-gram additions of sodium fluoride[b]	19.34

[a] Calcd., 19.32 ml.
[b] Fusion made at highest temperature attainable from blast lamp and prolonged until bottom of Pyrex flask sagged to spherical shape. Regular dithizone extraction then made prior to titration.

Decomposition of Sample. To determine under what conditions losses of thallium could be effected, 25-ml. aliquots of the standard thallium solution (1.996 mg. of thallium) were evaporated carefully to about 5 ml. and then were subjected to various treatments that might be used in everyday laboratory practice. After fuming with 1 ml. of concentrated sulfuric acid, the thallium content was determined by titration (Table V).

To make the error as noticeable as possible, the conditions employed in these tests were deliberately more extreme than usual laboratory practice would require. However, the results indicate that the temperature is the critical factor, its maximum permissible value depending on the type of anion present. (Some loss is indicated in test 9, but this is probably a small mechanical loss caused by the rather severe experimental conditions rather than volatilization.) Samples to be decomposed by acid treatment should be treated with hydrochloric and nitric acids in a covered beaker at a temperature just high enough to cause a gentle boil but not high enough to cause the sides of the beaker to dry or to cause too much evaporation before the addition of sulfuric acid and evaporation to fumes. The addition of sulfuric acid at the outset, if possible, would be even more desirable. Hydrofluoric acid in moderate quantities has not been observed to result in loss of thallium and should be used with siliceous material.

If much insoluble material is left from this acid treatment, it should be dissolved by pyrosulfate fusion to recover the thallium it contains. (On certain materials rich in antimony, over 50% of the thallium was found in the acid-insoluble residue.) As treating residues a second time is not desirable if a large volume of work is to be handled, this laboratory has made a practice of making the original decomposition by pyrosulfate fusion. Such treatment not only assures complete decomposition of most types of samples encountered but eliminates the concern over thallium losses that may occur with more volatile acids. However, when samples larger than 1 or 2 grams are taken for analysis, an acid decomposition would eliminate the large quantity of additional salts resulting from the fusion and might be a more suitable procedure. Such a decomposition might also be desirable in the treatment of high siliceous material where the use of hydrofluoric acid is necessary.

Final Check on Method. As a check on the final method as a combination of individually developed parts, a known quantity of thallium was evaporated to fumes with sulfuric acid, and a 1-gram sample of pyrite known to contain no thallium was added. After addition of sodium sulfate, the sample was fused and carried through the complete method as described. The data in Table VI show that the method yields completely satisfactory results.

SUMMARY

An accurate semimicrodetermination of thallium has been developed. Among the conditions for maintaining a stoichiometric reaction between thallium and iodide, the most important is the thallium-iodide ratio. The effects of starch, ultraviolet light, acidity, volatilization of iodine, and extraneous metals on the titration are discussed.

The separation of thallium by extraction with a chloroform solution of dithizone has been investigated, and the effects of cyanide concentration and pH on the extraction have been determined.

The volatility of thallium compounds under certain conditions common to everyday laboratory practice has been observed, and some methods of decomposing thallium ores that can be used without volatilization losses have been determined.

ACKNOWLEDGMENT

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The authors wish to acknowledge the assistance of L. C. Hendricks and J. R. Ross in the preliminary experimental work. They

Table VI. Analysis of Synthetic Samples

Pyrite Sample	Tl Added	Titration, 0.001011 *N*	Tl Found
Gram	%	*Ml.*	%
1	0.0000	0.00	0.0000
1	0.0200	1.94	0.0200
1	0.1996	19.29	0.1993

are particularly indebted to S. F. Ravitz for assistance in preparing the manuscript and offering suggestions.

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UNIVERSAL MICROAPPARATUS

Filtration, Extraction, Reflux, Distillation, Homogenization, Centrifugation, and Drying in the Same Apparatus

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One simple microapparatus alone can process small amounts of solid material through a series of common unit operations without vessel-to-vessel transfer and resulting high percentage loss of material. For radioactive tracer work, the apparatus presents a means of extended processing in a closed system. Other advantages are discussed.

FOR many years the trend in laboratory practice has been toward the micro scale. Microtechniques are not only time-saving and effort-saving, but become indispensable when only very small amounts of material are available for study. A special impetus has been contributed by isotopic tracer studies which demand the manipulation of very small amounts. Yet a persistent difficulty with microtechniques has been the percentage losses that are necessarily involved when small amounts of material are transferred from one vessel to another for different treatments. Apparently the ideal solution of this difficulty is the elimination of transfer by providing a single apparatus in which all necessary treatments can be carried out conveniently and efficiently. Substantial progress toward this goal has been accomplished by the new universal microapparatus.

An original form of this apparatus is shown in Figure 1. An improved form, shown in Figure 2, assumes the general shape of the combustion and carbon dioxide volatilization apparatus described by Allen, Gest, and Kamen (*1*) and attributed to the original design of H. A. Barker (*4*). The improved form differs from the Barker apparatus by the inclusion of a special disk assembly (Figure 4) that effectively adapts the admirable Barker design to a universal apparatus. To date this new apparatus has been successfully tested by processing plant material without transfer through filtration, extraction, reflux, distillation, homogenization, and centrifugation; and has been so employed in the rapid carbohydrate analysis of plant material and in the closed system processing of radioactive compounds.

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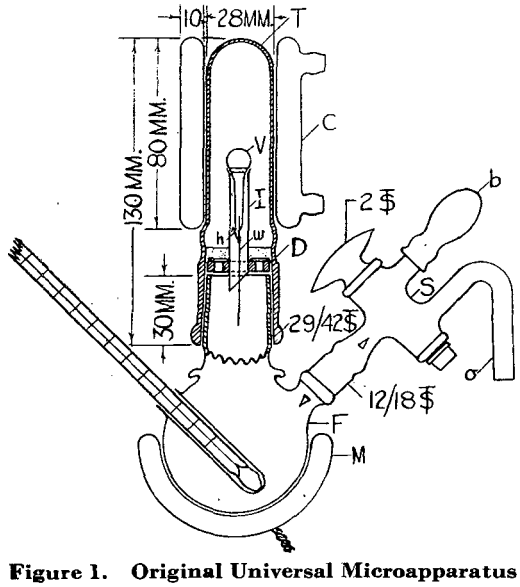

Figure 1. Original Universal Microapparatus

APPLICATIONS

Centrifugation and Homogenization. Tube *T* (Figures 1 and 2) serves as a rugged centrifuge tube which will fit any standard 50-ml. centrifuge cup. It serves as a homogenizing tube when a rotating motor-driven pestle (Figure 3) is inserted after the manner of Potter and Elvehjem (*7*, *8*).

Filtration. The disk assembly, detailed in Figure 4, includes the resin disk, *D*, which is tapered to fit snugly within the standard-taper joint of tube *T*. A disk of filter paper, *p*, is cemented, *c*, along its circumference to the resin disk and slightly overlaps the central hole into which it is tightly engaged by the insertion of the tapered inlet tube, *I*. When the disk assembly is slipped firmly into position within the neck of a tube which contains the suspension, slurry, or homogenate to be filtered, and the 100-ml. flask, *F* (Figure 1), or the midpiece, *M*, and second tube, T^1 (Figure 2), are attached, and the apparatus is inverted to a position illustrated in Figure 1 or 2, filtration proceeds through the paper disk and the six small holes in the resin disk.

The apparatus can frequently simplify refiltration procedures. In conventional filtrations, the initial filtrate passing through a fresh filter medium may be turbid although subsequent filtrate comes through clear. Refiltration is required, involving disassembly of apparatus, filtrate transfer, and rinsings. With the new arrangement, it is necessary merely to invert the apparatus, letting the turbid filtrate return to tube *T* via the inlet tube, then inverting again for refiltration.

Vacuum Filtration. When the apparatus is assembled, there is included the small glass valve, *V*, which has previously been ground into the top of the inlet tube to permit a tight seal. This valve carries a small Chromel wire hook, *h*, to be hooked over the top of the inlet tube (Figure 2) when the valve is not in use, and a straight wire, *w*, to permit convenient reseating of the valve. The stopcock assembly, *S*, is attached to the side arm and vacuum is applied through the outlet tube, *o*. In practice air leakage around the valve is frequently great enough to require

the alternate admission of air to the apparatus to restore the pressure in tube T and re-evacuation—a simple manipulation.

(If D is well seated, friction alone can hold it in position despite the stress caused by evacuation of the lower portion of the apparatus.)

Extraction. Valve V, if present, is hooked out of the way, and the entire apparatus is evacuated. The glass condenser, C,

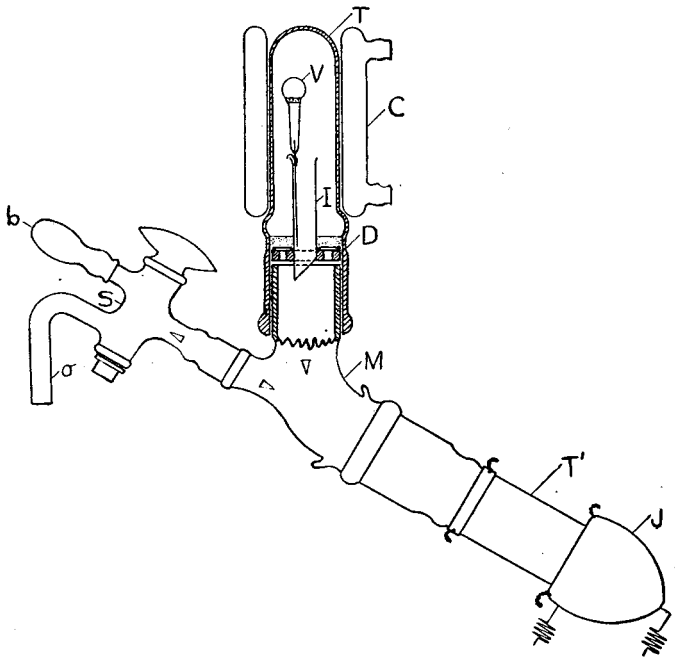

Figure 2. Improved Universal Microapparatus

is slipped over the tube. The flask (Figure 1) is heated with a Glas-Col heating mantle, or tube T' (Figure 2) is heated with a special jacket, J; regulation of temperature is accomplished by a variable transformer. The internal pressure can be checked occasionally by turning the stopcock to connect the system with the small rubber bulb, b, which should remain collapsed. Rarely does difficulty arise due to excess pressure within the system. Extraction proceeds as the extractant boils, passes up through the inlet tube, condenses in T, and percolates back through the material collected on D.

The apparatus permits unobscured observation of the material being extracted. In the extraction of green leaf material, the green color can be seen to pass as a descending zone, simulating an eluting chromatographic column. This feature provides a convenient means for judging completeness of extraction.

The apparatus eliminates the need for successive washings of residual material after filtration, a procedure that leads to an undesirably large volume of filtrate plus washings, and often requires subsequent concentration. With the new apparatus it is more convenient to "wash" the residue by simple extraction, whereby no volume increase is involved.

For subsequent applications, the improved form of the apparatus (Figure 2) is recommended.

Reflux. The application of the apparatus to simple reflux is obvious. However, let it be assumed that a material has just been extracted in the apparatus and it next is required to reflux it in intimate contact with a boiling liquid. The appropriate liquid is admitted, and the apparatus is momentarily inverted to let the liquid run into tube T, then inverted again so as to entrain the liquid about the inlet tube in T. Gentle swirling, performed while the apparatus is held in hand, serves to loosen up the material collected on the disk and bring it into suspension again. The apparatus is inverted once more, condenser C and heating jacket J are slipped into place, and the apparatus is in final position for reflux as sketched in C of Figure 5.

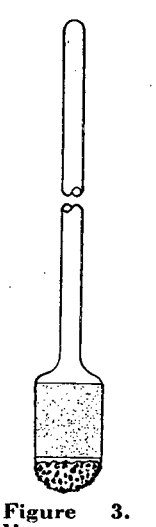

Figure 3. Homogenizing Pestle

The apparatus can frequently alleviate bumping problems. Considerable difficulty was initially encountered in attempting to boil plant homogenates in relatively narrow vessels at atmospheric pressure, for bumping invariably threw material from the vessels. The new apparatus, after evacuation with a water aspirator, is gradually heated while the lower tube is gently shaken (conveniently done by holding the lead wires to the heating jacket). Although mild bumping initially occurs, continued shaking and the gradual readmission of a little air into the system through the stopcock readily bring the suspension to a smooth boil; thereafter further manipulation is unnecessary.

Should initial bumping be inadvertently allowed to throw some solid material up through the inlet tube, I, advantage is taken of the feature discussed under filtration to wash it back into the lower tube.

Distillation. If the apparatus is disposed in the position sketched in D of Figure 5, simple distillation may take place from one tube to another. In this application the apparatus becomes essentially that described by Allen, Gest, and Kamen and attributed to Barker. These authors volatilized carbon dioxide under vacuum from one tube, using a gentle heat, and absorbed it in alkali solution contained in the other tube. Under increased heating actual distillation satisfactorily proceeds under vacuum. This arrangement has been applied to concentrate an aqueous solution of C^{14} glucose to a sirup, the last portion of water condensing on the walls of the tube being driven over by the heat from a flexible heating jacket (Figure 6) wrapped about the tube. In this particular case, the stopcock was opened to continuous evacuation in the latter stages.

Although distillation is preceded by an application in which the disk assembly is used, the apparatus need not be taken apart to remove the disk assembly, which will not interfere.

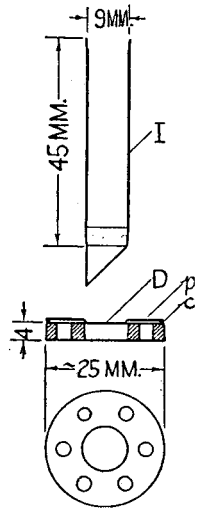

Figure 4. Disk Assembly

Drying. One tube may contain the material to be dried, the other may contain a suitable drying agent. After evacuation, the flexible heating jacket (Figure 6) may maintain the material at a suitable temperature as indicated by a thermometer placed between heater and jacket. In this case the apparatus becomes an efficient Abderhalden drying pistol.

In all applications the improved form of the universal microapparatus can be conveniently supported by a single three-fingered clamp gripping the midpiece. The tubes and stopcock assembly are held to the midpiece by springs engaging hooks. A metal collar carrying hooks is used with the tubes, in order that permanently attached hooks may not interfere with their application as centrifuge tubes. The condenser is slipped over the upper tube. The end heating jacket is quickly positioned by a long thin spring engaging the two hooks on the jacket and looping over one finger of the clamp, and the flexible heating jacket can be secured about a tube with spring-type clothespins. Thus the apparatus and its accessories form a compact system which is conveniently assembled, disassembled, or otherwise manipulated, either in a clamped position or while held in hand.

RAPID CARBOHYDRATE ANALYSIS

The following brief summary illustrates how the apparatus has been employed in an actual procedure. In conjunction with the

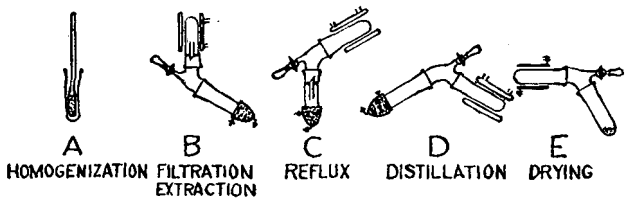

Figure 5. Positions of Apparatus for Different Applications

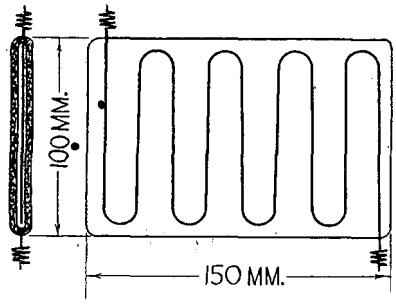

Figure 6. Flexible Heating Pad

extraction scheme of Hassid, McCready, and Rosenfels (*3*), and a modification of the colorimetric method of Morris (*6*) using Dreywood's anthrone reagent (*2*), the apparatus permits a rapid means of carbohydrate analysis.

Rapid disintegration of a fresh sweet potato leaf (weight 300 to 500 mg.) is accomplished by homogenizing it with 1 ml. of 80% alcohol in tube *T*, using a special pestle (Figure 3), to the end of which sharp silicon carbide teeth have been cemented. The pestle is rinsed down with 4 ml. more of 80% alcohol. Homogenization and rinsings require only 5 minutes. The universal apparatus is then assembled, and the leaf residue is filtered and extracted therein for 1 hour. The tube of alcohol extract is removed.

A second tube, containing 5 ml. of acid alcohol (4 ml. of concentrated sulfuric acid stirred into 500 ml. of 95% alcohol), is attached, and the residue is brought into suspension again as previously described, and boiled for 15 minutes (to "solubilize" the starch), followed by inversion of the apparatus and extraction for 15 minutes. The efficiency of this extraction, which replaces the repeated alcohol washings required by the conventional procedure, is proved by the inability of the extracted residue to redden litmus.

A third tube, containing 5 ml. of water, is substituted for the acid alcohol tube, and boiling (1 hour) and extraction (15 minutes) are repeated. The tube of aqueous starch extract is removed. Finally, the acid alcohol and water treatments are repeated. (Slow water extractions are sometimes encountered due to the gelatinous nature of the acid-treated plant residue.)

For analysis, appropriate aliquots of the 80% alcohol extract (soluble sugars), the water extract (starch), and standard glucose solutions are directly pipetted into separate tubes, to each of which are added 5 ml. of anthrone reagent (1 gram of anthrone dissolved in 1 liter of concentrated sulfuric acid, stirred into 50 ml. of water, and cooled). The mixtures are heated in a boiling water bath for 10 minutes, cooled, and read in a Klett-Summerson colorimeter using a 540-mμ filter. Under the conditions described, a straight-line plot of Klett reading against weight of standard glucose was obtained up to at least 200 micrograms of glucose (corresponding to a Klett reading of approximately 450).

The plant material is processed without transfer from the original tube, followed by prompt determination of the total carbohydrate contents of the soluble sugar and starch extracts without "clearing" and hydrolysis treatments.

PROCESSING OF RADIOACTIVE COMPOUNDS

The apparatus has been employed to isolate C^{14} glucose from a photosynthesizing leaf. By providing a means of processing radioactive material through a series of treatments in a closed system, the apparatus minimizes contamination of the atmosphere with radioactivity. It thereby diminishes the health hazard and aids in maintaining a satisfactorily low background count in the laboratory. The closed system feature is especially advantageous when high temperature treatments such as extraction, reflux, and distillation are involved. When the apparatus is cooled after such a treatment, any volatile material residual in the apparatus may be removed before disassembly by evacuation of the apparatus through suitable traps (dry ice and carbon dioxide absorption traps).

CONSTRUCTION DETAILS

Sufficient details are given in the figures and text to clarify the construction of the glass portions of the apparatus. The ground zones of the inner 29/42 standard-taper joints are cut off to an approximate length of 30 mm., so that the disk assembly may be accommodated; the bulges around the necks of the tubes should be small enough to permit insertion into centrifuge cups. Further details are required for some of the accessories.

Disk Assembly (Figure 4). The resin disk, *D*, is molded inside the ground zone of an outer 29/42 standard-taper joint which has been packed with firm clay, and the clay surface is flattened down to a level approximately 35 mm. below the lip of the joint. A glass tube (9 mm. in diameter), centered inside the tube, is inserted into the clay; and six smaller tubes (3 mm. in diameter) are inserted symmetrically about the central tube. Tubes, clay, and joint are lubricated with silicone grease. A thick mass of asbestos fiber and thermosetting resin is packed in about the tubes to a thickness of about 4 mm.; the flat unrounded end of a glass rod serves to tamp the upper surface flat. (The resin employed is Plastitool, manufactured by Calresin Corporation, Culver City, Calif., and is a thick sirup into which a small amount of catalyst solution is stirred before use.) The assembly is baked in an oven at 50° to 60° C. for 0.5 to 1 hour, which treatment sets the resin mixture to a hard mass, resistant to most solvents and chemical agents. The upper and lower surfaces of the disk are sanded and, for greater durability, varnished with resin and baked again.

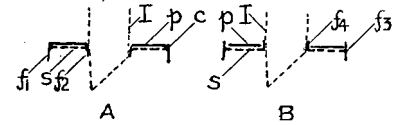

Figure 7. Metal Disk Assemblies

The grade of paper chosen for the disk assembly will vary with the nature of the material being filtered. For leaf homogenates the rather thick porous paper used in extraction thimbles has proved very satisfactory, and clear green filtrates have been consistently obtained. Each paper disk, *p*, is given a preliminary coat of resin over an approximate 2-mm. zone about its circumference and baked. A second coat is applied, the paper disk is pressed firmly against the resin disk (a glass slide employed with spring-type clothespins is convenient for this purpose), and the assembly is baked again.

A metal disk similar to those sketched in Figure 7 might be superior to the resin disk. In *A* there is shown a metal disk with an outer flange, f_1, to fit into the standard-taper joint and an inner flange, f_2, to take the inlet tube, *I*. In order not to entrain liquid about the disk when the apparatus is inverted, f_2 should contain a slot or hole. The disk presents the sieve area, *s*. The paper disk, *p*, is cemented on as before. In *B* is shown a disk with a double outer flange, f_3, and an inner upwardly disposed flange, f_4. If cut large enough, *p* can fit snugly between the flanges, eliminating the need for cementing.

Linch (*5*) has suggested that *D* be made of sintered glass and sealed inside the inner 29/42 standard-taper member of adapter (midpiece) *M* flush with the top, and tube *I* be either fitted as shown (Figure 2) or sealed directly to the porous disk to make a rigid assembly. Although the author believes the use of separate disks favors flexibility in choice of filters for different filtration and extraction requirements, the suggested modification provides convenient and positive means of seating and removing the disk.

End Heating Jacket (*J*, Figure 2). A jacket of asbestos cloth is sewed tightly about the end of a tube using glass thread, and the free edges of the cloth are whipped with the thread. Approximately 40 cm. of No. 20 Chromel wire are wrapped about the asbestos and securely sewed on with glass thread, and the whole is coated with a thick paste of asbestos fiber and water glass (sodium silicate solution), and allowed to dry. This jacket will usually operate at 10 volts or less.

Flexible Heating Jacket (Figure 6). About 90 cm. of No. 20 Chromel wire are sewed onto a piece of asbestos cloth in the

manner illustrated. A similar piece of asbestos cloth is overlaid and the jacket is sewed together.

Homogenizing Pestle (Figure 3). The end of the pestle is roughened with emery paper, coated with a layer of phenolic thermosetting resin, and dipped into sharp silicon carbide particles, 6/30 grit. One half hour in an oven at 50° to 60° C. firmly sets the particles in place.

ACKNOWLEDGMENT

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Organic Onium Compounds as Inorganic Analytical Reagents

Detection of Bismuth and Cobalt

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The action of various organic ammonium, phosphonium, arsonium, stibonium, oxonium, sulfonium, selenonium, telluronium, and iodonium ions on iodide and thiocyanate complexes of a number of metals was investigated. On the basis of the reactions observed new tests are proposed for the detection of bismuth and cobalt.

THE organic reagents used most frequently in the detection and determination of metals are compounds that contain in the molecule acidic groups, the hydrogen atoms of which may be replaced by metal atoms. Such reagents ionize to give organic anions which react with metal ions to form simple or inner-complex salts. Compounds that ionize to give organic cations have been used less frequently as analytical reagents. Because many metals readily form complex anions, the possibility of employing organic cations for the detection and determination of these metals through salt formation is evident.

The particular organic cations which have come into use as reagents for metals are predominantly nitrogen compounds. Tetraphenylarsonium ion stands out as an important exception. Willard and Smith (*21*) have shown that this cation may be used for the determination of mercury, tin, cadmium, zinc, and rhenium. Smith (*18*) has recently employed this same cation for the determination of thallium. Dwyer, Gibson, and Nyholm (*9*) have reported the use of methyl aryl arsonium compounds in the detection of bismuth and cadmium and in the detection and estimation of cobalt. Possible analytical applications of onium compounds of elements other than nitrogen and arsenic have not thus far been reported.

DETECTION OF BISMUTH

Alkyl ammonium salts, aniline, pyridine, quinoline, certain alkaloids, and a large number of other nitrogen bases are known to react with bismuth in the presence of iodide to give colored, insoluble reaction products (*20*). That this type of reaction is not limited to nitrogen compounds is indicated by the fact that alkyl sulfonium iodides react with bismuth in a similar manner (*2, 13*). The observations cited are perhaps best interpreted by assuming that in each case reaction has occurred between an organic cation and a bismuth-containing anion or anions.

In beginning a survey of the reactions of onium ions, various organic onium compounds of nitrogen, phosphorus, arsenic, antimony, oxygen, sulfur, selenium, tellurium, and iodine were tested with respect to their reactivity toward BiI_4^-. (The reactions here described are attributed to BiI_4^-, which is taken as the probable formula of the predominant bismuth anion present in a bismuth solution containing excess iodide. No evidence is presented to support this formula.) The fourteen potential reagents which were investigated are listed in Table I. Optimum conditions for reaction, limits of sensitivity, and interfering effects of foreign ions were determined for each of the compounds tested. Cinchonine was included because it is a spot test reagent commonly used in bismuth detection (*10*) and could therefore serve as a convenient standard of reference. Tetramethylammonium bromide (*13*), trimethylsulfonium iodide (*2*), and triethylsulfonium iodide (*13*) had been observed to react with bismuth, but no study had been made of the possible analytical applications of the reactions.

The limits of sensitivity given in Table I are in terms of micrograms of bismuth per drop (0.05 ml.) of test solution. The reactions were carried out on spot test paper (Schleicher and Schüll No. 601), following the technique recommended by Feigl (*10*).

The compounds tested were found to be remarkably similar in their behavior toward BiI_4^- in that all reacted to give insoluble orange or red-orange products.

Preliminary studies indicated that from considerations of specificity and sensitivity the aryl sulfonium, triphenylselenonium, tetraphenylphosphonium, tetraphenylarsonium, and tetraphenylstibonium compounds were the best of the reagents tested. In

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Table I. Limits of Sensitivity of Onium Compounds in Detection of Bismuth

Compound[a]	Formula	Reference[b]	Sensitivity, γ
2-Phenyl-β-naphthopyrylium chloride (0.03% solution in methyl alcohol)	$(C_{19}H_{13}O)Cl$	(7, 12)	0.1
Trimethylsulfonium iodide (5% solution)	$(CH_3)_3SI$	(19)	0.2
Triethylsulfonium iodide (5% solution)	$(C_2H_5)_3SI$	(16)	0.2
Triphenylsulfonium chloride (5% solution)	$(C_6H_5)_3SCl$	(6)	0.1
m-Xylyldiphenylsulfonium chloride (5% solution)	$C_8H_9(C_6H_5)_2SCl$	(6)	0.1
Triphenylselenonium chloride (5% solution)	$(C_6H_5)_3SeCl$	(17)	0.1
Triphenyltelluronium iodide (saturated solution)	$(C_6H_5)_3TeI$	(14)	0.2
Diphenyliodonium iodide (saturated solution)	$(C_6H_5)_2II$	(15)	0.3
Di-p-chlorophenyliodonium bromide (saturated solution)	$(ClC_6H_4)_2IBr$	(15)	0.2
Tetramethylammonium bromide (5% solution)	$(CH_3)_4NBr$		0.3
Tetraphenylphosphonium bromide (1% solution)	$(C_6H_5)_4PBr$	(8)	0.1
Tetraphenylarsonium bromide (1% solution)	$(C_6H_5)_4AsBr$	(3, 4)	0.1
Tetraphenylstibonium bromide (saturated solution)	$(C_6H_5)_4SbBr$	(5)	0.1
Cinchonine (1% solution in dilute nitric acid	$C_{19}H_{22}N_2O$		0.1

[a] Aqueous solutions unless otherwise indicated.
[b] Literature references relate to procedures for preparing compounds.

the detection of bismuth these compounds are fully as sensitive and somewhat more selective than cinchonine. They form stable aqueous solutions. (Tetraphenylarsonium chloride is commercially available through the Hach Chemical and Oxygen Company, Ames, Iowa.)

In making the detailed interference studies described below only two reagents, triphenylsulfonium bromide and tetraphenylarsonium bromide, were used. Tetraphenylarsonium bromide was made by the procedure of Blicke and Monroe (3). Triphenylsulfonium bromide was prepared as follows:

Phenylmagnesium bromide and diphenylsulfoxide were heated under reflux in a benzene ether mixture at 65° to 68° C. for 27 hours. The mixture obtained was hydrolyzed with 40% hydrobromic acid. The aqueous phase was separated and the triphenylsulfonium bromide was extracted from it with chloroform. The crude material obtained upon evaporation of the chloroform was crystallized from acetone and chloroform by addition of diethyl ether. Triphenylsulfonium bromide, a white, crystalline, water-soluble substance, was obtained in 32% yield. The compound melts with decomposition at 286–288° C. (uncorrected). It was identified by bromine analysis. Calculated for $(C_6H_5)_3SBr$: 23.28% bromine. Found: 23.21, 23.30% bromine.

Procedure for Detection of Bismuth. One drop of the slightly acid test solution is treated on spot test paper (or spot plate) with one drop of triphenylsulfonium (or tetraphenylarsonium) reagent solution and one drop of 10% potassium iodide. In the presence of bismuth an orange precipitate forms.

The acidity need not be carefully controlled, but because the sensitivity of the test falls off slightly at high acidity the hydrogen ion concentration is kept below 0.1 *M*, or below 0.01 *M* if very small amounts of bismuth are being sought.

Interferences. The behavior of elements other than bismuth under the conditions of the bismuth test was investigated by experiments in which a variety of inorganic ions were treated with triphenylsulfonium bromide and tetraphenylarsonium bromide reagents. Stock solutions (containing 1 mg. per ml. or 10 mg. per ml. of the test constituent) were prepared using the substances shown in Table II. Test solutions were made at the time of use by diluting the stock solutions. In making tests 1 ml. of the test solution containing 500 micrograms of the test constituent was treated with excess potassium iodide and reagent solution (3 drops of 10% potassium iodide plus 3 drops of 0.05 *M* triphenylsulfonium bromide or 0.04 *M* tetraphenylarsonium bromide). The extent of interference in the bismuth test was investigated by repeating each test in the presence of bismuth, using solutions in which bismuth and the foreign test constituent were present at concentrations of 5 and 500 micrograms per ml., respectively.

The ions that interfere are listed in Table III.

Cd(II), Hg(II), and In(III) gave evidence of reacting with the onium reagents (in the presence of excess iodide). Cd(II) and Hg(II) gave precipitates with both reagents. The Cd(II) precipitates were white, the Hg(II) precipitates cream white. In(III) gave a white precipitate with tetraphenylarsonium bromide but gave no reaction with triphenylsulfonium bromide. It was found, however, that bismuth could be readily detected with either reagent, even when Cd(II), Hg(II), or In(III) was present in 100-fold excess [5 micrograms of Bi(III) to 500 m crograms of Cd(II), Hg(II), or In(III)]. For this reason Cd(II) Hg(II), and In(III) are not classed as interferences. Tetraphenylarsonium iodide is insoluble and may separate, as a white crystalline precipitate, from solutions to which tetraphenylarsonium and iodide have been added.

The extent of interference by the commoner anions was further investigated by making the bismuth test in the presence of a 1000-fold gravimetric excess (5 micrograms of bismuth to 5000 micrograms of test anion per ml.) of each of the following anions added in the form of ammonium or sodium salts: Ac^-, NO_3^-, SO_4^{--}, HSO_3^-, F^-, Cl^-, Br^-. Of these anions, only fluoride interfered slightly when present in 1000-fold excess but showed no interference when present in 100-fold excess.

Ions which oxidize I^- to I_3^-—e.g., ferric and cupric—interfere in the bismuth test, the brown color of I_3^- tending to obscure the oniumiodobismuthite. To eliminate this interference the I_3^- is reduced with bisulfite. The test is made on spot test paper. One drop of test solution is treated with one drop of onium (triphenyl-

Table II. Composition of Stock Solutions

Nitrates
Li(I), Na(I), K(I), NH_4^+, Be(II), Mg(II), Ca(II), Sr(II), Ba(II), Y(III), La(III), Th(IV), Cr(III), U(VI), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Ag(I), Zn(II), Cd(II), Hg(I), Al(III), Ga(III), In(III), Tl(I), Pb(II), Bi(III)

Chlorides
Zr(IV), Ru(III), Rh(III), Pd(II), Pt(IV), Au(III), Hg(II), Sn(II), Sn(IV), As(III), Sb(III)

Sulfates
Ce(III), V(IV), Fe(II)

Alkali or ammonium salts
Acetate, fluoride, bromide, bisulfite, arsenate, selenate, selenite, tellurite

Table III. Interferences in Detection of Bismuth

Test Constituent[a]	KI + Ph_3SBr	KI + Ph_4AsBr	Interference in Bi Test Prevented by Addition of
Sn(II)	Light yellow ppt.	Light yellow ppt.	NH_4F
Pb(II)	Yellow ppt. (PbI_2)	Yellow ppt. (PbI_2)	$NaHSO_3$
Sb(III)	Yellow ppt.	Yellow ppt.	NH_4F
Cu(II)	Brown (I_3^-) plus ppt.	Brown (I_3^-) plus ppt.	$NaHSO_3$
Ag(I)	Light yellow ppt. (AgI)	Light yellow ppt. (AgI)	
Au(III)	Light brown ppt.	Brown ppt.	$NaHSO_3$
Hg(I)	Gray ppt.	Gray ppt.	
Tl(I)	Yellow ppt. (TlI)	Yellow ppt. (TlI)	
Fe(III)	Brown (I_3) plus ppt.	Brown (I_3) plus ppt.	$NaHSO_3$ (or NH_4F)
Pt(IV)	Dark reddish-violet ppt.	Reddish-violet ppt.	$NaHSO_3$
Ru(III)	Brown ppt.	Brown ppt.	
Pd(II)	Dark brown ppt.	Dark brown ppt.	
Se(IV)	Brown (I_3^-) + ppt. (Se)	Brown (I_3^-) + ppt. (Se)	$NaHSO_3$
Se(VI)	Brown (I_3^-) + ppt. (Se)	Brown (I_3^-) + ppt. (Se)	$NaHSO_3$
Te(IV)	Brown ppt.	Brown ppt.	NH_4F

[a] See Table II for compounds used in preparation of test solutions.

Table IV. Detection of Cobalt

Onium Compound[a]	KOCN	KSCN	KSeCN
2-Phenyl-β-naphthopyrylium chloride (in methyl alcohol)	−	−	−
Trimethylsulfonium iodide	−	−	−
Triethylsulfonium iodide	−	−	−
Triphenylsulfonium chloride	+	+++	++
m-Xylyldiphenylsulfonium chloride	+	+++	++
Triphenylselenonium chloride	+	+++	++
Triphenyltelluronium chloride	+	++	++
Diphenyliodonium chloride	−	++	+
Tetramethylammonium bromide	−	−	−
Cinchonine (in dilute HNO_3)	−	−	−
Tetraphenylphosphonium bromide	+	+++	++
Tetraphenylarsonium bromide	+	+++	++
Tetraphenylstibonium bromide	−	+	−

[a] Compounds dissolved in water unless otherwise indicated.

sulfonium or tetraphenylarsonium) reagent, one drop of 10% potassium iodide, and one drop of 10% sodium bisulfite. In the presence of certain oxidizing substances—for example, selenite and selenate—better results are obtained if the bisulfite is added to the test solution before addition of the other reagents.

Bisulfite is effective also in preventing interference by Pt(IV), Au(III), and Pb(II), the colored reaction products of platinum, gold, and lead being converted by bisulfite to colorless substances. If platinum or lead is present, the bisulfite is added to the test solution before addition of the onium reagent and iodide.

Sb(III) reacts under the conditions of the bismuth test to give a bright yellow voluminous precipitate. The formation of the yellow antimony compound can be prevented by the addition of fluoride. In the presence of antimony, the bismuth test is made on a spot plate, 1 or 2 drops of 10% ammonium fluoride solution being added to the test solution before addition of iodide and onium reagent. An excessive amount of fluoride is to be avoided, as it decreases the sensitivity of the bismuth test.

Dark brown substances precipitate when certain onium ions are added to solutions containing the iodotellurite ion. The formation of these precipitates can be prevented by adding ammonium fluoride, and carrying out the bismuth test in the same manner as when antimony is present.

Sn(II) interferes slightly in the bismuth test, forming a light yellow precipitate with iodide and triphenylsulfonium or tetraphenylarsonium ion. This interference also is prevented by addition of fluoride.

By making use of ammonium fluoride a few micrograms of bismuth are readily detected even in the presence of a 100-fold excess of Sb(III), Te(IV), or Sn(II).

It was observed that a number of the compounds precipitated by iodide and onium ions—tetraphenylarsonium iodobismuthite, tetraphenylarsonium iodoantimonite, tetraphenylarsonium iodotellurite, tetraphenylarsonium iodopalladite, and triphenylsulfonium iodotellurite —are readily soluble in chloroform. This circumstance makes possible the separation of certain elements from aqueous solution by chloroform extraction of their onium iodide complexes. A procedure for the determination of tellurium is based on the chloroform extraction of the highly colored metaxylyldiphenylsulfonium iodotellurite followed by colorimetric analysis of the chloroform solution (*11*).

DETECTION OF COBALT

When triphenylsulfonium ion is added to a solution containing Co(II) and excess thiocyanate ion, a blue, chloroform-soluble substance precipitates. The formation and chloroform extraction of the blue substance are made the basis of a sensitive test for cobalt. The limit of identification under the conditions described is about 0.05 microgram of cobalt per test drop. This corresponds to a dilution limit of 1 to 1,000,000. These limits were determined by using a volume of chloroform equal to the volume of the test solution. Greater sensitivity is achieved by using a volume of chloroform less than that of the test solution.

Some aryl onium ions other than triphenylsulfonium give similar color reactions. Cyanate may be used in place of thiocyanate, but the test is less sensitive. Selenocyanate was also tried but was found to possess no apparent advantage over thiocyanate. In Table IV are summarized results of tests carried out using equal concentrations of cobalt with excess cyanate, thiocyanate, or selenocyanate and various onium reagents. The plus signs indicate approximate, relative intensities of the blue color in the chloroform layer under essentially identical test conditions. Attention is directed to the fact that the oxonium, ammonium, and alkyl sulfonium compounds which were used gave no observable reactions. The tests with these compounds moreover remain negative even when large amounts of cobalt are present.

The blue substances that precipitate from the cobalt solutions result, presumably, from interaction of the onium ions with cobaltous thiocyanate complex (or complexes). Chemical analyses on one of these substances, the *m*-xylyldiphenylsulfonium compound, gave results in agreement with the formula, $[C_6H_3(C_6H_5)_2S]_2Co(SCN)_4$. The analytical data are given in Table V.

Table V. Analysis of *m*-Xylyldiphenylsulfonium Cobaltothiocyanate

	Found	Calculated for $[C_6H_3(C_6H_5)_2S]_2CO(SCN)_4$
% C	60.51, 60.50	60.46
% H	4.45, 4.52	4.38
% N	6.50	6.41
% Co	6.70, 6.67	6.74

Analyses by Huffman Microanalytical Laboratories, Denver, Colo.

Procedure for Detection of Cobalt. One half milliliter or less of the slightly acid test solution is treated in a small test tube with 1 or 2 drops of triphenylsulfonium (or tetraphenylarsonium) reagent solution and 1 or 2 drops of 10% ammonium thiocyanate. A few drops of chloroform are added and the mixture is shaken vigorously. In the presence of cobalt the chloroform layer becomes blue.

Maximum color development is obtained at a pH of approximately 3.5. The acidity, however, is not critical.

Table VI. Interferences in Detection of Cobalt

Test Constituent[a]	NH_4SCN + Ph_3SBr	NH_4SCN + Ph_4AsBr[b]	Interference in Co Test Prevented by Addition of
U(VI)	Yellow ppt. soluble in $CHCl_3$	Yellow ppt. soluble in $CHCl_3$	NH_4F
Fe(III)	Deep red ppt. somewhat soluble in $CHCl_3$	Deep red ppt. readily soluble in $CHCl_3$	NH_4F
Ru(III)	Dark purplish-red ppt. very slightly soluble in $CHCl_3$	Dark purplish-red ppt. slightly soluble in $CHCl_3$	$NaHSO_3$ (or $Na_2S_2O_3$)
Pd(II)	Orange ppt. readily soluble in $CHCl_3$	Orange ppt. readily soluble in $CHCl_3$	$Na_2S_2O_3$
Pt(IV)	Yellow ppt. soluble in $CHCl_3$	Yellow ppt. soluble in $CHCl_3$	$NaHSO_3$ (or $Na_2S_2O_3$)
Cu(II)	Red-brown ppt. soluble in $CHCl_3$	Red-brown ppt. soluble in $CHCl_3$	KI + $Na_2S_2O_3$
Bi(III)	Yellow ppt. soluble in $CHCl_3$	Yellow ppt. soluble in $CHCl_3$	NH_4F

[a] See Table II for compounds used in preparation of test solutions.
[b] Tetraphenylarsonium thiocyanate is insoluble and separates as white precipitate from solutions to which SCN^- and Ph_4As^+ have been added. It dissolves in $CHCl_3$ to give colorless solution.

Interferences. Interference experiments for the cobalt reaction were carried out by a procedure similar to the one that was followed in studying interferences in the bismuth reaction. One milliliter of the test solution containing 500 micrograms of the test constituent (Table II) was treated with excess thiocyanate and onium reagent (3 drops of 10% ammonium thiocyanate and

3 drops of 0.05 *M* triphenylsulfonium bromide or 0.04 *M* tetraphenylarsonium bromide solution). Approximately 1 ml. of chloroform was added and the mixture was shaken. The extent of interference in the cobalt test was investigated by repeating each of the tests in the presence of cobalt, using solutions in which cobalt and the foreign test constituent were present at concentrations of 5 and 500 micrograms per ml., respectively.

Triphenylsulfonium bromide and tetraphenylarsonium bromide when used as reagents in the cobalt test were found to be subject to the same interferences.

Zn(II) and Sn(IV) give slight negative interference, preventing full development of the blue color. This interference is minimized by using a large excess of thiocyanate and onium reagent.

Masking interference was observed in the presence of U(VI), Fe(III), Ru(III), Pd(II), Pt(IV), Cu(II), and Bi(III). The nature of these interferences and methods of preventing them are indicated in Table VI.

The very pronounced interference by Fe(III) and slight interferences by Bi(III) and U(VI) are easily prevented by adding to the solution to be tested 1 or 2 drops of 10% ammonium fluoride. (The bismuth thiocyanate complex unlike BiI_4^- is readily decomposed by fluoride.)

Cu(II) interference is prevented by reducing to Cu(I) with iodide. Thiosulfate serves to remove the iodine formed. One drop each of 10% potassium iodide and 10% sodium thiosulfate are added to the solution to be tested.

The feasibility of employing the onium-thiocyanate-chloroform extraction procedure for the separation and colorimetric determination of cobalt has been demonstrated (*1*). Studies on applications of the procedure are in progress.

CONCLUSIONS

Triphenylsulfonium and tetraphenylarsonium chlorides and bromides used in conjunction with iodide are particularly suitable reagents for the detection of bismuth. They are fully as sensitive as the commonly used cinchonine reagent and are somewhat less subject to interferences. They possess an advantage over cinchonine of being water-soluble. The same compounds used with thiocyanate serve as sensitive and, under properly controlled conditions, highly specific reagents for the detection of cobalt.

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Determination of Hydrogen Peroxide in Small Concentrations

A Spectrophotometric Method

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A sensitive method of peroxide analysis which allows not only the detection but the quantitative determination of hydrogen peroxide in concentrations as small as 10^{-6} molar is described. The determination of the optimum wave length for the spectrophotometric determination is described. A typical standardization curve is given.

A SENSITIVE method of peroxide analysis, which provides not only for the detection but the quantitative determination of hydrogen peroxide in concentrations as small as 10^{-6} molar, involves the oxidation of iodide ion to iodine, in the peroxide solution to be determined, and the estimation of the free iodine thus formed by measurement of its light absorption on a spectrophotometer. In order to make the rather slow iodide ion–peroxide reaction go rapidly to completion, use is made of the catalytic action of molybdic acid discovered by Brode (*1*) and employed by Kolthoff (*2*). The method is limited to solutions which do not contain other constituents that either oxidize iodide ion or absorb in the same spectral range as triiodide ion.

A standardization curve is obtained by diluting an approximately 1.0 molar solution of hydrogen peroxide to about 0.01 molar and then analyzing this solution accurately by thiosulfate determination of liberated iodine according to standard procedure.

Table I. Transmittance and Absorption Values[a]

Wave Length	% Transmittance	% Absorption
8.00 ml. of 1.31 × 10^{-6} molar hydrogen peroxide. 1.00 ml. of 1 *M* sulfuric acid, 10^{-3} *F* ammonium molybdate. 1.00 ml. of 1 *F* potassium iodide.		
3200	74.0	26.0
3300	68.9	31.1
3400	60.5	39.5
3500	56.5	43.5
3600	58.3	41.7
3700	65.0	35.0
3800	73.5	26.5
3900	81.0	19.0
4000	87.1	12.9
8.00 ml. of 6.35 × 10^{-6} molar hydrogen peroxide. 1.00 ml. of 1 *M* sulfuric acid, 10^{-3} *F* ammonium molybdate. 1.00 ml. of 1 *F* potassium iodide.		
3200	86.5	13.5
3300	83.5	16.5
3400	78.2	21.8
3500	75.8	24.2
3600	76.8	23.2
3700	80.7	19.3
3800	85.7	14.3
3900	90.0	10.0
4000	93.1	6.9
8.00 ml. of 3.17 × 10^{-6} *M* hydrogen peroxide. 1.00 ml. of 1 *M* sulfuric acid, 10^{-3} *F* ammonium molybdate. 1.00 ml. of 1 *F* potassium iodide.		
3200	95.0	5.0
3300	93.0	7.0
3400	90.1	9.9
3500	88.1	11.9
3600	88.8	11.2
3700	90.8	9.2
3800	93.2	6.8
3900	95.5	4.5
4000	97.0	3.0
8.00 ml. of 1.31 × 10^{-6} *M* hydrogen peroxide. 1.00 ml. of 1 *M* sulfuric acid, 10^{-3} *F* ammonium molybdate. 1.00 ml. of 1 *F* potassium iodide.		
3200	97.0	3.0
3300	96.2	3.8
3400	95.1	4.9
3500	95.0	5.0
3600	95.0	5.0
3700	96.0	4.0
3800	97.4	2.6
3900	98.4	1.6
4000	99.1(?)	0.9(?)

[a] All figures are given for 20 Å. band width around wave length indicated.

The solution is then diluted accurately to the range of concentrations given in Table I, and in each case to 8.00 ml. of the resultant solution are added 1.00 ml. of a 1 *M* sulfuric acid, 10^{-3} *F* ammonium molybdate solution, and 1.00 ml. of a 1 *F* potassium iodide solution, the latter freshly prepared. The absorption spectra were in all cases determined within 20 minutes after addition of the potassium iodide solution to avoid any oxidation of the iodide ion by the oxygen of the air, and were determined over the range 3000 to 4000 Å. in order to find the optimum wave length for use with an unknown. In making an actual determination on a solution of unknown peroxide concentration it is necessary to make only one reading at the selected wave length.

All the per cent transmittance values in Table I and Figures 1 and 2 are based upon the value of the blank (8 mm. of distilled water plus reagents as above) taken as 100%. The readings taken on the various solutions in the spectrophotometer (Beckman quartz cell spectrophotometer) remain nearly constant for an hour or more after the fresh iodide reagent is added. After longer periods, however, there is a considerable increase in absorption shown by the solution due to air oxidation of the iodide ion.

It is seen from Figure 1 that the maximum per cent absorption is obtained at a wave length of 3500 Å. This wave length was accordingly taken as the standard for the subsequent analyses performed.

In order to test the applicability of Beer's law, log I/I_0 (at 3500 Å.) for the various hydrogen peroxide (I_3^-) concentrations used for standardization are tabulated in Table II and plotted in Figure 2.

Because the solutions corresponding to these concentrations were diluted from 8 to 10 ml. by the addition of the reagents, the concentrations actually existent in the absorption cell at the time of measurement are actually only eight tenths of those shown. In Figure 2 it is seen that log I/I_0 versus concentration does not give exactly a straight line, showing that Beer's law is not obeyed, in accord with Winther (*3*). The plot in Figure 2 serves, however, as a standard curve for this procedure of analysis.

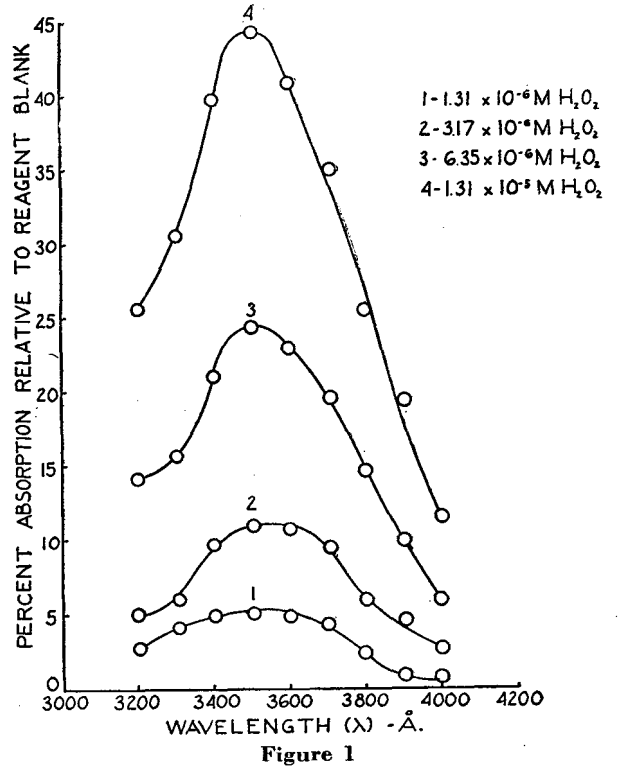

Figure 1

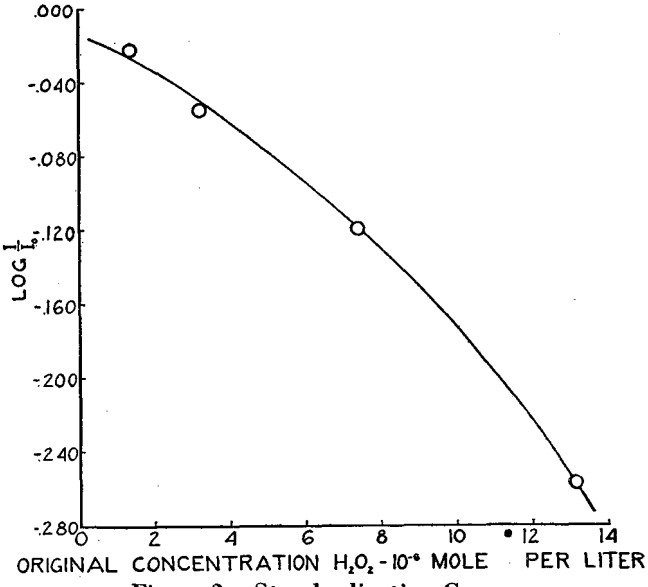

Figure 2. Standardization Curve

Table II. Concentrations Used for Standardization

I/I_0	Log I/I_0	H_2O_2 (I_3^-) Molarity (× 10^6)
0.565	−0.248	13.1
0.758	−0.120	6.35
0.881	−0.055	3.17
0.950	−0.022	1.31

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Spectrochemical Determination of Beryllium in Microquantities

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A spectrochemical procedure has been developed, using a conventional direct current arc, for the quantitative determination of beryllium in amounts as low as 0.04 microgram of beryllium per liter of urine. The sample is prepared by a phosphate precipitation to which aluminum is added to act as an internal standard. The amount of beryllium is determined by comparing the density of Be 2348.6 Å. to Al 2321.6 Å. In addition to the determination of beryllium in urine, the method has been successfully applied to tissue and air samples.

THE determination of beryllium in biological material has become important to industry. Cholak (*1*) has described a method which gave a sensitivity of 6.25 micrograms of beryllium per liter of urine. This procedure was used in preliminary survey work and found to be of insufficient sensitivity for many urine samples. Because it utilized only 20% of the sample on the electrode, experimental work was begun in order to use the entire sample. Utilizing the phosphate precipitation procedure (*1*), with some modification, for the concentration and separation of the beryllium, a satisfactory concentrate has been obtained for the spectrographic method. Quantitative data have been obtained which show that 0.04 microgram of beryllium per liter of urine can be determined with accuracy, and 0.02 microgram of beryllium per liter of urine can be qualitatively detected. Cholak (*2*) has more recently published a quantitative procedure utilizing the cathode layer technique which, from the data given, yields a quantitative sensitivity of 0.10 microgram of beryllium per liter of urine, and a qualitative detection of 0.025 microgram per liter of urine.

The method described makes use of a conventional direct current arc with the powdered sample placed on the anode. Chemical preparation involves wet ashing, precipitation, centrifuging, and drying of all the beryllium (with phosphates) from a 50-ml. sample of urine which is then burned to completion in the arc.

SPECIAL REAGENTS

All the reagents specified are analytical grade.

Aluminum Internal Standard (1.50 mg. of aluminum per ml.). Dissolve 0.750 gram of metallic aluminum in approximately 10 ml. of 1 to 1 hydrochloric acid and make up to 500 ml. with distilled water.

Ammonium Nitrate Wash. Dissolve 10 grams of ammonium nitrate in water and make up to 1000 ml. Adjust to pH 7.5 with ammonium hydroxide.

Calcium Phosphate Solution. As described by Cholak (*1*), dissolve 2.5 grams of calcium carbonate (calcite) in just sufficient concentrated hydrochloric acid (specific gravity 1.19) to effect solution (about 7 ml.), add 2.5 grams of ammonium phosphate, and make up the solution to 100 ml. with distilled water. If a precipitate forms, redissolve by adding hydrochloric acid drop by drop.

Standard Beryllium Solution. Dissolve a weighed amount of fused metallic beryllium in a small amount of 1 to 1 hydrochloric acid and dilute to volume with 1% hydrochloric acid in distilled water. Make further dilutions to required concentration in 1% hydrochloric acid, using a microburet and volumetric flasks. Take extreme care to avoid contamination during these dilutions. A standard solution containing 0.02 microgram of beryllium per milliliter has been used.

Synthetic Urine. Sodium chloride, 170.5 grams; potassium chloride, 63.5 grams; calcium chloride hexahydrate, 31.5 grams; magnesium chloride hexahydrate, 20 grams; sodium dihydrogen phosphate monohydrate, 37.5 grams; dissolved in and diluted to 1000 ml. with 10% by volume nitric acid.

CHEMICAL PROCEDURE

To 50 ml. of urine in a 300-ml. tall-form Pyrex beaker add 20 ml. of nitric acid and 5 ml. of sulfuric acid. Heat this mixture about 3 hours on the hot plate, during which time most of the liquid is evaporated. Heating is not continued to dryness. When the liquid begins to turn a brown color, add 1 ml. of nitric acid. Continue heating at approximately the same rate until the solution again begins to turn brown and then add 1 ml. of nitric acid. Repeat this procedure until a clear, colorless solution results. Greater amounts of sulfur trioxide fumes are evolved each successive time it becomes necessary to add nitric acid. If spattering occurs during the addition of the nitric acid, reduce the heat slightly. After the solution appears colorless, continue additions of 1-ml. portions of nitric acid at approximately 15-minute intervals until the evolution of sulfur trioxide fumes becomes noticeably less but has not entirely stopped. Do not take to dryness but drive out most of the sulfuric acid. Add 1 ml. of perchloric acid in such a way as to rinse down the sides of the beaker and heat the mixture to a frothing condition over a separate burner. As soon as the liquid has cooled enough to prevent spattering, add approximately 40 ml. of hot distilled water and transfer the clear solution to a 50-ml. graduated Pyrex centrifuge tube where it is allowed to cool. The centrifuge tubes which seem best are those with a short conical bottom.

Add 2 drops of phenol red and then concentrated ammonium hydroxide until the first evidence of color change. Then add dilute ammonium hydroxide (1 to 5) until the first definite precipitation of phosphates occurs. The beryllium also precipitates at this time. Complete precipitation of the phosphates is not necessary. The aim should be to precipitate an amount which will yield a correct final precipitate weight; with a little experience this can be done. In some urine samples, the amount of phosphate is so small that no precipitate occurs, even though sufficient ammonium hydroxide has been added to turn the indicator very red. In such cases, add a few drops of nitric acid to make the solution slightly acid again. Add 0.5 ml. of the calcium phosphate solution and again add dilute ammonium hydroxide until precipitation occurs. After precipitating the phosphates, add 1 ml. of the aluminum internal standard solution from a microburet; it immediately forms a precipitate. Centrifuge for 10 minutes at 1800 r.p.m., the radius of rotation being 18.75 cm. (7.5 inches) at the bottom of the centrifuge cups. Pour off supernatant liquid. Add approximately 20 ml. of ammonium nitrate wash; swirl to break up and wash the precipitate. Centrifuge and pour off supernatant liquid. Repeat this wash once.

Transfer precipitate to a weighed 10-ml. fused silica crucible with the aid of a small volume of water, and place in oven at 130° C. until sample is completely dried. This may take from 2 to 3 hours. Weigh and adjust weight, if necessary, to between 40 and 60 mg. of precipitate by addition of the appropriate amount of calcium phosphate solution, and dry again. Scrape the precipitate loose from the walls and bottom of the crucible with a small spatula and transfer this precipitate to a prepared spectrographic carbon.

SPECTROGRAPHIC EQUIPMENT

A large Gaertner quartz spectrograph has been used in this method. Accessory equipment includes a Westinghouse Rectox rectifier to provide a direct current source, a Leeds & Northrup

nonrecording microphotometer, and developing equipment of Applied Research Laboratories. A neutral filter and miscellaneous cutters for shaping electrodes are also used. The electrodes are regular grade spectroscopic carbons of the National Carbon Company.

SPECTROGRAPHIC PROCEDURE

The lower electrodes used to contain the sample are cut into 5-cm. (2-inch) lengths and one end is cupped with a special cutter (*4*). It has been found experimentally that the dimensions of the electrode must be held to close tolerances in order to secure the best results. The specifications are as follows:

Outside diameter of electrode	0.250 ± 0.002 inch
Inside diameter of electrode	0.210 ± 0.001 inch
Drill angle	48 degrees
Depth to bottom of cup	0.180 ± 0.020 inch
Length of electrode	2.0 inches

The upper electrode is 2 inches long and 0.25 inch in diameter. It is tapered and hemispherically tipped with a cutter to give an end with 0.06-inch radius. This shaped electrode withstands the high amperage and will not burn rapidly.

The electrodes are mounted on the arc stand and a 2-mm. gap set between them. No adjustment of gap length is made during the arcing period. The optical arrangement consists only of a 40% neutral filter between the source and the slit. The slit is 20 microns wide and 3 mm. long.

The sample is burned to completion in a conventional 220-volt direct current arc at 16 amperes. In order to prevent spattering, the arc is started at 3 amperes until fusion of the sample has taken place, which requires approximately 30 seconds. The current is then rapidly increased to 16 amperes. The length of burning time is approximately 150 seconds; no pre-arc is used.

The spectrum is recorded on Eastman Kodak Company S.A. No. 1 plates in the wave-length region 2290 to 3100 Å. The plates are developed in D-19 (Eastman Kodak) for 3 minutes, hardened in chrome alum, and fixed in x-ray fixer. The temperature is maintained at 70° ± 1° F. A two-line method, similar to that proposed by the Aluminum Company of America (*3*), is used for plate calibration, except that an iron spectrum is substituted for aluminum.

DISCUSSION

Early in this investigation, it was recognized that beryllium is a refractory element that would not readily volatilize from the electrode until near the end of the arcing period. It was desirable, therefore, to choose an internal standard that would have similar characteristics and remain in the arc until the sample burned to completion. Aluminum has been found satisfactory for this purpose. A moving plate study indicates that the density of the beryllium line increases with time and does not reach its maximum value until near the end of the arcing period. The aluminum line has a final density value little different from its value during the entire arcing period.

The line pair used for the analysis is Be 2348.6 Å. and Al 2321.6 II Å. Background is measurable in this region but no corrections have been necessary to obtain the required accuracy. Referring to Figure 1, the difference in slope of the two analytical curves may be partially attributed to the neglect of background corrections in the low concentration region. However, a straight-line relationship is obtained throughout the analytical range as shown. The technique as described is capable of quantitatively determining 0.04 microgram of beryllium per liter of urine and qualitatively detecting 0.02 microgram of beryllium per liter (corresponding to 0.001 microgram of beryllium in the arc). The standard deviation for a single determination at 0.020 microgram is ±0.004 or 20% of the amount present. This is based on a statistical analysis of over 50 standard samples, using all results obtained. The standard deviation suggests the need for duplicate determinations, which has been the practice.

Certain precautions must be followed to secure optimum results. The standard solution containing 0.02 microgram of beryllium per milliliter must be acid to prevent adsorption on the glassware. Experimental data have been obtained on solutions with and without the acid addition. Consistently low results have been found for the nonacidified solutions. Statistical

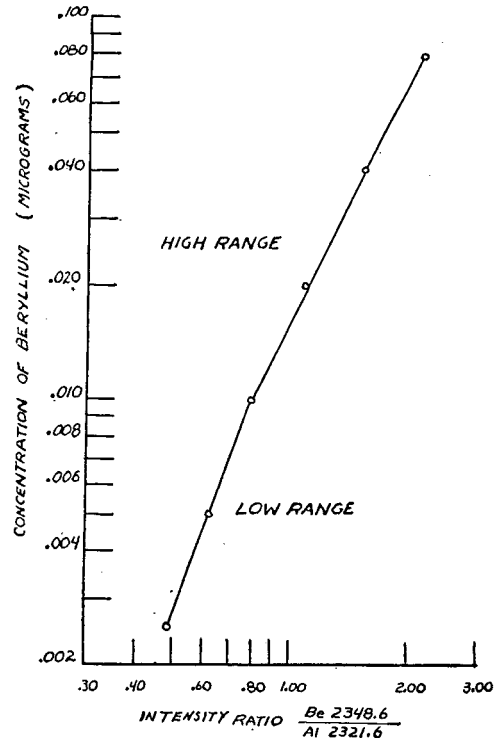

Figure 1. Analytical Curve for Determination of Beryllium

evidence on the acidified standard has shown that stability exists over a period of several months.

The final precipitate as obtained from the chemical procedure is made up of refractory materials which are difficult to burn to completion. Experimental work has shown that the electrode shape and size, as mentioned above, must be met in all particulars. The weight of the dried precipitate must also be held to a critical range, and should preferably lie between 40 and 50 mg. As much as 60 mg. can be burned successfully if the material is free from large amounts of sodium salts. Because the sodium salts are soluble, they can be removed by washing. Data have been obtained to show that losses of beryllium due to washing with ammonium nitrate wash are negligible. Excessively long burning times are to be avoided, as the background will then interfere with the lower end of the analytical range.

When working with such small quantities of beryllium, the contamination problem becomes paramount. Glassware which has contained strong solutions of beryllium should not be used for very dilute solutions. Pyrex does not contain detectable amounts of beryllium, but porcelain crucibles were found to contain small amounts and fused silica crucibles have been used instead. Contamination of samples during collection or preparation must be avoided by adopting extreme precautions. The spectrographic carbons have not shown the presence of beryllium.

The technique, as described, has been successfully applied to urine samples, lung tissue, skin, and dust samples.

Lung tissue and skin are ashed by placing 10 grams of tissue in a 250-ml. Pyrex beaker and proceeding as previously described for urine samples. For lung tissue or other samples which contain little or no phosphate, it is necessary to add 0.8 ml. of the calcium phosphate solution before trying to precipitate the phosphates with dilute ammonium hydroxide. Analysis of dust samples collected by the electrostatic dust and fume sampler indicates the method of analysis is satisfactory for these samples. The dust is washed from the stainless steel sampling tubes with water and a policeman, 5 ml. of hydrochloric acid are added, and the solution is heated to dissolve the beryllium compounds. Most, but not all, of the hydrochloric acid is driven off and the solutions made up to a definite volume with water. A measured portion of

this is transferred to a centrifuge tube and water added to make approximately 40 ml. of solution. Two drops of bromothymol blue are used as an indicator instead of phenol red; 0.8 ml. of calcium phosphate solution is added and the analysis is continued as previously described. Higher concentrations than the range specified are easily analyzed by using aliquot parts or by dilution. An aliquot can conveniently be taken while the sample is in liquid form in the centrifuge tube prior to the precipitation of phosphates.

The above procedure does not depart from standard techniques used by the analytical chemist, and elaborate optical adjustments for the spectrographic procedure are avoided. This enables the spectrographer to perform the analyses on the prepared samples with speed and facility. The short time required for setup is particularly advantageous in an industrial laboratory where many different problems are encountered.

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All-Metal Needle Valve of the Hershberg-Southworth Type

Committee for the Standardization of Microchemical Apparatus
Division of Analytical and Micro Chemistry, AMERICAN CHEMICAL SOCIETY

AL STEYERMARK, *Chairman*
H. K. ALBER, V. A. ALUISE, E. W. D. HUFFMAN, J. A. KUCK, J. J. MORAN, AND C. O. WILLITS

THE three-way stopcock originally described by Pregl (*2–6*), for use between the precision nitrometer and the combustion tube in the micro-Dumas determination, has been used by most chemists engaged in microanalytical work up to the present time. Hershberg and Southworth (*1*) described a needle valve to be used in place of the three-way stopcock, which gave the operator better control of the rate of flow of gas into the micronitrometer. The one disadvantage of this needle valve was the breakage of the

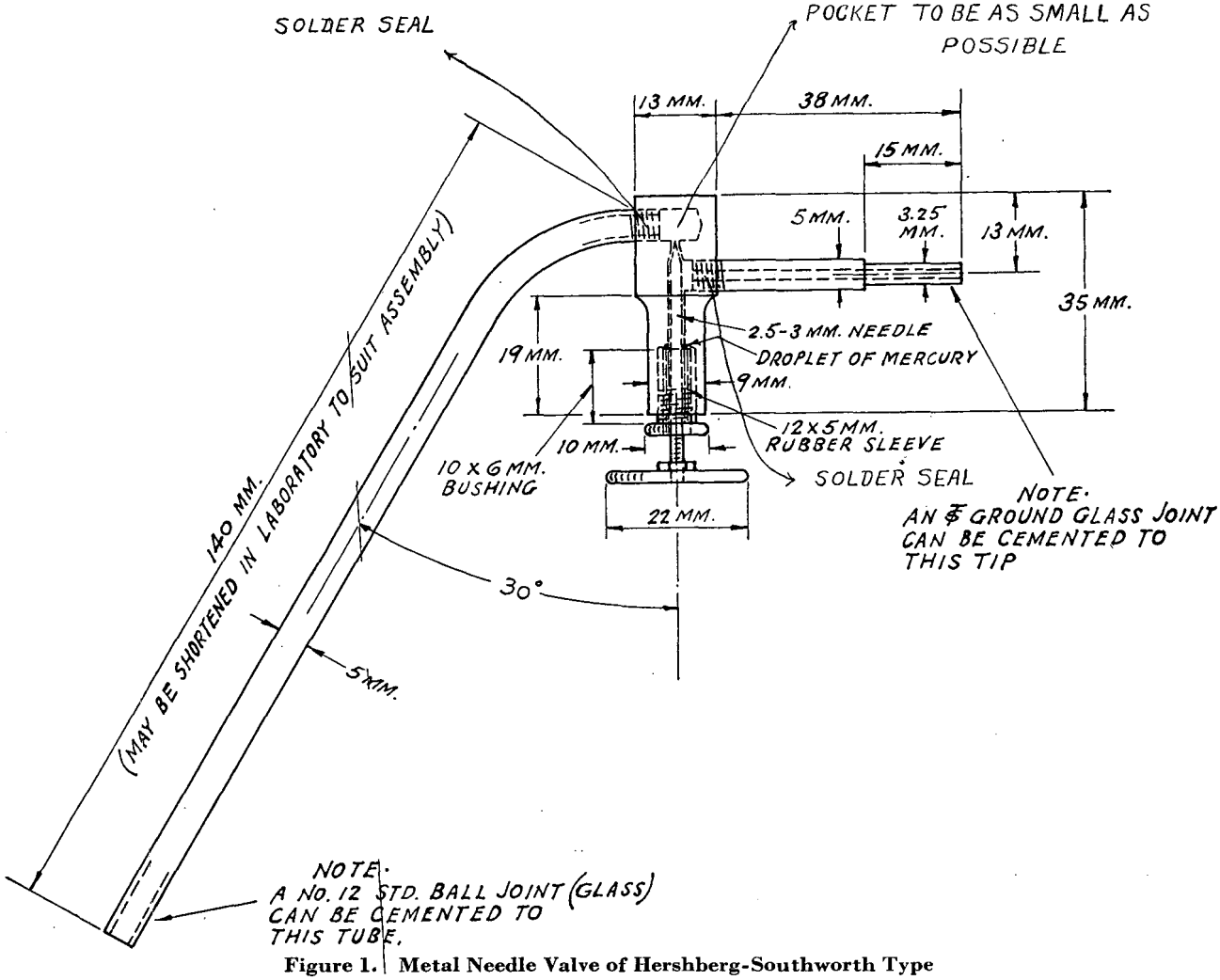

Figure 1. Metal Needle Valve of Hershberg-Southworth Type

glass seat which frequently occurred at the place of contact with the metal needle.

This disadvantage has been eliminated by making the apparatus entirely of stainless steel. The details of construction are given in Figure 1. Gas enters the valve at the right through the horizontal capillary, passes through the conoidal seat, and leaves by way of the downward slanting tube at the left. Flow of gas through the valve is precisely regulated by turning the needle provided with a large knurled disk. Fittings are made gas-tight by means of a rubber sleeve, but a droplet of mercury is used at the point indicated as an additional precaution against leakage. The mercury is introduced through the horizontal capillary. The valve must not leak with working pressures up to and including 10 pounds per square inch.

The all-metal needle valve described here is lighter in weight than the glass-metal type which requires the use of several grams of mercury as a seal.

The diameters of the tubes follow the recommended specifications for the Dumas stopcock (*5*, *6*).

ACKNOWLEDGMENT

The sketch of the needle valve (Figure 1) was made by H. T. Adams, Kimble Glass Division of Owens-Illinois Glass Company, Vineland, N. J.

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NOTES ON ANALYTICAL PROCEDURES . . .

Large-Capacity Laboratory Vacuum Sublimation Apparatus

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DURING the course of certain investigations carried out in this laboratory, it was necessary to purify relatively large batches of different materials by sublimation. Inasmuch as none of the laboratory sublimators described in the literature (*1–8*) seemed adequate for the purpose, construction of the apparatus described below was undertaken. It has proved extremely useful and convenient for subliming from 1 to 100 grams of material.

As shown in Figure 1, the unit consists of a horizontal jacketed Pyrex sublimation chamber, *H*, heated by a resistance wire, *B*, wound around its entire length. Through the chamber runs a straight Pyrex tube, *I*, parallel with the axis of and considerably longer than the chamber, which serves as a water-cooled condenser. The ends of the chamber are closed by means of rubber stoppers, *C*, which are bored, somewhat off center, to allow passage of the condenser. A glass tube, *D*, passing through one stopper is used for the vacuum connection. The condenser tube tapers just beyond the point where it emerges from the assembly to permit easy removal of the stopper.

The condenser tube is made longer than the chamber in order that it may be clamped rigidly in position at the end of the apparatus. In preparation of the apparatus for operation, *H* and outer jacket *G* are removed while the left-hand stopper is held in place on the condenser tube. The material to be sublimed is distributed throughout the length of the sublimation chamber and the apparatus is reassembled by carefully sliding the chamber and jacket into place using the supporting guide rails, *J*, to prevent contact of the condenser with the crude material. A thermometer, *K*, is placed in the chamber in contact with the material to be sublimed. The unit may then be placed in operation by application of a vacuum and heating of the resistance coil, *B*, by means of a variable electric current.

Upon completion of the sublimation, the water hose and electrical leads are disconnected and the right-hand stopper is carefully loosened and removed. The left-hand stopper is loosened by gentle motions of the chamber, after which the chamber is slipped off the condenser, the rails guiding its path so that no contact is made between the purified and the crude materials. A clean paper may then be spread below the condenser tube and the product may be scraped off and collected on the paper.

The temperature range and the control obtainable in an apparatus of the dimensions indicated in Figure 1 are shown in Table I.

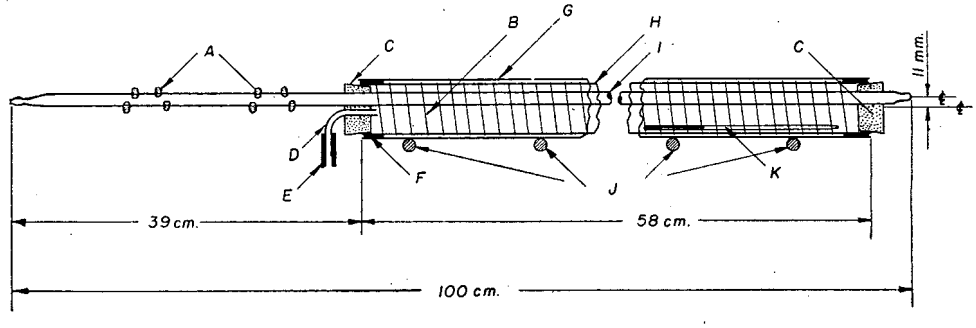

Figure 1. Diagram of Sublimation Apparatus

A. Clamps, 2-fingered
B. Heating coils, 23 feet of Nichrome wire, B. and S. No. 22
C. Rubber stopper, size 12
D. Glass tube, 8 mm. o.d.
E. Vacuum pump connection
F. Asbestos tape
G. Glass tube, 70 mm. o.d., 65 mm. i.d.
H. Glass tube, 60 mm. o.d., 54 mm. i.d.
I. Glass tube, 14 mm. o.d.
J. Supporting rails
K. Thermometer

Table I. Variation of Temperature with Voltage

Volts	Temperature, ° C.
30	52
40	76
50	105
60	136
70	166
80	191
90	219
100	248
110	275

The notable features combined in the apparatus are its relatively large capacity, the simplicity of its construction and operation, its extreme flexibility, and the short distance between evaporating and condensing surfaces which it makes possible.

Portions up to 100 grams of a wide variety of organic compounds, including such representative materials as 8-hydroxyquinoline (8-quinolinol), *p*-aminophenol, pentachlorophenol, and *p*-fluorobenzoic acid, have been sublimed successfully in the unit described.

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Analysis of Residual Gas in Electron Tubes with the Light Spectrograph

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THE presence of even a small amount of gas in a finished high-vacuum electron tube is generally sufficient cause for factory rejection of that tube. Unwanted gas can be introduced during the manufacturing process in many ways; unclean parts, improper breakdown of oxide-coated cathodes on exhaust, leaks, hydrogen absorption through metal parts, and poor tip-offs are typical causes of gassy tubes. If the composition of the unwanted gas is known, the gas source can often be found and a serious epidemic of gassy tubes prevented.

Few of the standard methods of gas analysis are applicable to the analysis of the gas in a finished tube. Standard methods of gas absorption in various reagents are ruled out because of the small size of the sample, which in many cases is less than 1 microliter. The difficulty of opening the finished tube and transferring the sample also rules out most methods. Most of the samples are too small for even the mass spectrograph.

The method that offers the most promise uses the light spectrograph or spectroscope. When the spectrograph is employed, it is first necessary to ionize the gas and obtain a visible discharge which can be focused on the slit of the spectrograph. The simplest way of accomplishing this step is by means of a spark discharge, such as can be obtained with a Tesla coil. The use of the spark coil, however, makes it difficult to obtain a concentrated beam of light because the discharge tends to flicker and be diffused rather than sharp.

In most cases, a more desirable source of light can be obtained by using a high-voltage transformer and applying voltage between two terminals of the tube. This procedure makes it generally possible not only to control the discharge but to focus the light accurately on the spectrograph slit with little difficulty.

A third way applies particularly to cathode-ray tubes and is by far the best when it can be used. In a completed cathode-ray tube, it is possible to use the cathode-ray gun to furnish a stream of high-speed electrons which will ionize the gas and give a concentrated discharge.

None of these procedures requires opening the tube and the gas can be analyzed without destroying the tube.

After a procedure for ionizing the gas has been selected, the next problem is the choice of a spectrograph. Initially, the author's analyses were made using a large Littrow Band L spectrograph, Eastman I-F plates (specially made for spectrographic work, with high sensitivity in the region of 4500 to 6800 Å. and medium contrast), a slit opening of 250 microns, the widest opening of the collimating lenses, and normally a 10-minute exposure. The spectrograph was adjusted to photograph the spectrum from 4500 to 6000 Å. Later, a Bausch & Lomb constant-deviation spectrometer was utilized, but because camera attachments were not available commercially, it was necessary to build a simple attachment, which takes a 2 × 2 inch plate. The assembled equipment is illustrated in Figure 1. The instrument has provisions for superimposing an iron spectrum on the gas spectrum for reference purposes.

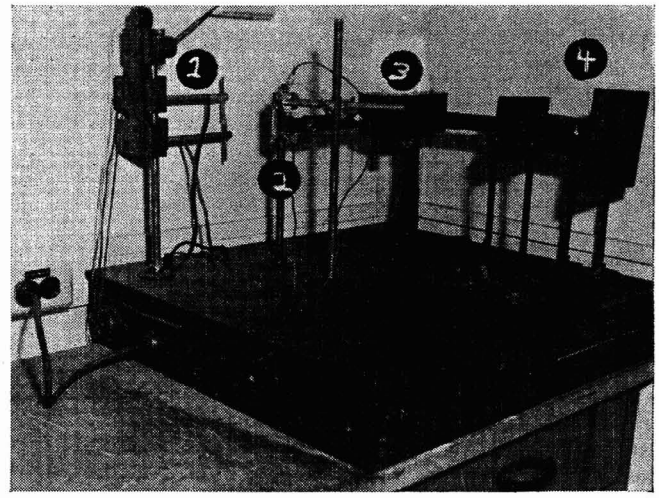

Figure 1. Assembled Equipment
1. Iron reference
2. Gassy tube
3. Spectroscope
4. Camera attachment

Standard comparison plates for the gases most commonly encountered were made by utilizing Geissler tubes filled with gas of known purity and ionizing the gas with a high-voltage source. Each standard plate contains the iron spectrum superimposed on a gas spectrum. Unknown samples tested in the same way are lined up by means of the iron references, making it possible to determine the identity of the unknown gases. After an operator becomes familiar with the spectrum of the more common gases, it is possible to use the instrument as a spectroscope and determine visually the composition of many gases. In case of doubt, comparison plates can be made.

One of the first problems solved by the use of this equipment was the determination of an unknown gas in several cathode-ray tubes. The electron beam was used to ionize the gas which was then identified as mercury. Further investigation revealed that operators of the exhaust machines had failed to fill several liquid-air traps, and mercury vapor from the mercury-diffusion pumps had therefore flooded the system. In another case, the presence of water vapor in cathode-ray tubes was shown to be caused by breakdown of overheated mica parts. Before the identification of the water vapor, such gassy tubes were called "air tubes" and much engineering time was consumed in a futile search for nonexistent leaks.

An epidemic of gas in a phototube type was shown to be air. The trouble was traced to microscopic holes in a metal part caused by improper spot welding.

Spectrographic analysis showed the presence of hydrogen in an expensive power tube type that became gassy after being electroplated. At first it was believed that the acid cleaning treatment prior to plating had caused leaks, but after the spectrographic analysis had been made, the source of trouble was quickly found to be due to diffusion of hydrogen through the steel during the cleaning and plating.

The spectrograph can also be used to check the purity of gases used in gas-filled tubes and to make special analyses of the gases released by suspected parts which have been sealed in dummy tubes and heated to drive off gas.

The chief limitation of the suggested method is that a minimum quantity of gas is required to provide the visual glow. Many tubes which are rejected as gassy have too little gas in them to permit analysis. Another limitation is that the method is purely qualitative and, therefore, cannot give quantitative results. There are several reasons for this; not only is the ionization of the gas dependent on its total pressure and the kinds of gases present, but the presence of one gas may suppress the ionization of another gas.

In spite of the limitations of the method, the light spectrograph has been found very useful in the manufacture of electron tubes.

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Distilling Apparatus for Production of Pure Water

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THE still described is an improvement of similar apparatus developed by the author. By changing the position of the product condenser and making other minor modifications it has been possible to provide for diversion of water that has been in contact with glass at high temperature away from the distillate, to reduce contact between distillate and glass to the minimum necessary for condensation, and to provide for preliminary degassing of the raw feed water.

The still shown in Figure 1 employs concentric reflux columns with take-off between the upper and lower parts somewhat similar to those used in two prior stills (*3*, *4*), but it embodies modifications in construction and alteration in sequence of functions which result in improved operation and performance.

The simplified still used at Bushnell General Hospital (*4*) was criticized by Liebig (*6*) because of contamination of distillate by extensive and prolonged contact at high temperature between the final product and the glass surfaces in the second stage column and in the hot outlet trap. In the present apparatus, the reflux from the degassing tube, which corresponds to the second stage in the older apparatus, is returned to the boiler instead of to the product; the hot vapor and distillate in the product condenser are in contact with only the absolute minimum area of glass surface required for condensation; and the condensate drips directly into a delivery tube where it is in contact with a minimum area of glass.

In the second stage of the old apparatus, the refluxed condensate was in contact throughout its course with vapor that carried a maximum load of gases and steam-distillable substances. The present apparatus removes these substances from the raw water in a countercurrent manner before it enters the boiling flask, so that the steam flowing over the product-condensing surfaces will contain the minimum attainable content of water-soluble volatile matter.

In the earlier stills, the starting material was distilled water. The present still, which occupies much less space on the bench than the one used at Wright Field (*3*), produces from tap water, a distillate nearly as pure and somewhat better than that obtained from the Bushnell still (*4*). The quality of the distillate is indicated by Tables I and II.

Table I. Properties of Product of Still and of Water from Other Sources

	Sample or Source	NH_4 Ion	Cl Ion	$KMnO_4$ Reduced, Ml.	Specific Conductivity	Equivalent NaCl, P.P.M.
I.	Product of still plus CO_2 (measured)[a]	No color	Clear no color	0.27	1.22×10^{-6}	0.8
II.	Tap water, Christ Hospital[b]	Pale yellow	Faint blue cloud	0.37	...	...
III.	Lab.-distilled water, Christ Hospital[c]	Yellow	Faint blue cloud	0.32	...	...
IV.	Conductivity water, plus CO_2[d]	...	...	...	1.00×10^{-6}	...
V.	Best conductivity water, Kohlrausch (at 18° C.)[e]	...	...	...	0.043×10^{-6}	...
VI.	Product of still, CO_2-free (estimated)[f]	...	...	...	Less than 0.3×10^{-6}	Less than 0.2

[a] Saturated with CO_2 on standing.
[b] Cincinnati water supply, from raw feed water of still.
[c] From regular supply of laboratory, produced by Stokes still.
[d] Typical value found in Department of Chemistry, University of Cincinnati, for "conductivity water" after exposure to air to saturation.
[e] Value accepted as best in literature, from Kohlrausch, quoted by Daniels *et al.* (*1*).
[f] Conductivity value for IV subtracted from I, assuming that, at these concentrations, equal amounts of CO_2 have proportional effect on conductivity and that equivalent of NaCl can be extrapolated beyond data of Getman (*2*).

OPERATION

The three principal parts of the column are the degassing tube, vapor return tube, and outer shell, arranged in the order named from center outward below the product condenser. This condenser and the delivery tube are located at the top of the vapor passage corresponding to the old first stage, instead of at the top of the second stage as formerly. A feed water control unit (not shown) may be improvised; the essential features are an open-top tube or vessel with connections from the effluent side of the waste vapor condenser and to the feed water inlet and an overflow to the sink above the latter.

The boiling flask is charged with about 200 ml. of distilled water saved from a previous run. With the feed water control unit set to overflow at a point below the top of the degassing tube, the heater is switched on and cooling water is started through the con-

Table II. Tests of Elimination of Added Contaminants

[10 grams of NaCl in boiling flask (approximately 1%). Readings in dial units of Klett-Summerson photoelectric colorimeter with yellow filter]

	Amount of Entrainment		
	Distillate from Tap Water	Distillate from 1% NaCl Soln.	Standard NaCl, 1 P.P.M.
Blank (tubes and water)	1	5	7
AgCl in acid solution	3	7	44
Minus blank	2	2	37
AgCl in buffered solution	5	6	46
Minus blank	4	1	39
Visible appearance	Clear	Clear	Distinctly milky

Expt.	In Feed Water, P.P.M.	B. In Boiler, P.P.M.	D. In Distillate, P.P.M.	Ratio D/B
	Distribution of 2-Naphthol			
1	...	1000	200	1/5
2	1000	1000	200	1/5
3	20 ml. of 100 p.p.m. during 30 mins.	1–2	0.2 (approx.)	Between 1/5 and 1/10
	Distribution of Phenol			
4	100	37	70	[a]
5	100	34	71	[b]
6	20	40	14	[c]

[a] With 75 turns of No. 26 Chromel A wire in degassing tube.
[b] With 100 turns of No. 16 copper.
[c] With 100 turns of No. 26 Chromel A.

densers. When the water in the flask is boiling vigorously, and steam has filled the column and reached the waste vapor condenser, the feed water control unit is raised to a previously determined level which has been found to increase the volume of water in the flask to 800 to 1200 ml. during a day's run (3 to 6 cm. above degasser). Raw feed water then enters the preheater and overflows into the top of the degassing tube.

Steam from the boiling flask is prevented from entering the degassing tube by water in the small trap at the bottom of the vapor return tube, and is forced to follow a single course through the column. The steam flows from the flask to the product condenser through the space between the vapor return tube and the outer shell and through the lower connecting tube. The cold finger of the condenser removes a fixed portion as product distillate, which drips directly into the inner end of the delivery tube. The remaining steam returns to the column through the upper connecting tube, and is conducted to the bottom of the degassing tube through the vapor return tube. The steam then flows upward in the degassing tube over the surface of the film of entering feed water, removing from it gases and volatile and steam-distillable substances, and carrying them out through the top of the column. The amount of steam involved in this scavenging function may be estimated by measuring the volume of reflux delivered from the side tube below the waste vapor condenser.

To terminate the run, the feed control unit is lowered and the heater and cooling water are shut off. The residue in the flask is discarded.

EVALUATION OF PERFORMANCE

Data in Table I are taken from analyses of the product of an earlier model, in which the raw water entered a small glass unit in the bottom of the boiling flask and was elevated to the top of the degassing tube by a centrally located air lift. Preheating of feed water took place in this pump in the boiler. The ratio of steam wasted to that condensed as product was about 1 to 2, but the air escaping from the top of the lift undoubtedly aided in flushing out gases. On the other hand, the air itself may have introduced some contaminants.

Data in Table II were obtained from the product of the present still as shown in Figure 1, operating at a ratio of rejected steam to take-off of about 1 to 1. Preheating occurs above the vapor return tube and adds appreciably to the reflux in this tube.

Measurement of electrical resistance for determination of conductivity (Table I) was made in an open beaker in a constant-temperature bath at 25° C., using dipping platinum electrodes in a conventional bridge circuit. The contents of the beaker were replaced with fresh portions of sample several times until constant maximum resistance was observed. In the absence of any drift toward lower resistance thereafter, it was assumed that the sample had reached saturation with carbon dioxide at the usual ambient partial pressure.

Ammonium ion (including amines or ammonia derivatives capable of producing color with the reagent, if present) was determined qualitatively several times over a period of weeks. An equal small amount of Nessler's reagent (about 0.2 ml.) was added to equal samples (about 4 ml.) from each of the three sources. The relative contamination shown is typical. In all cases, the product of the still was negative to the eye and the other samples showed visible color.

For the detection of chloride ion, dilute silver nitrate in acid solution was added dropwise to a small sample.

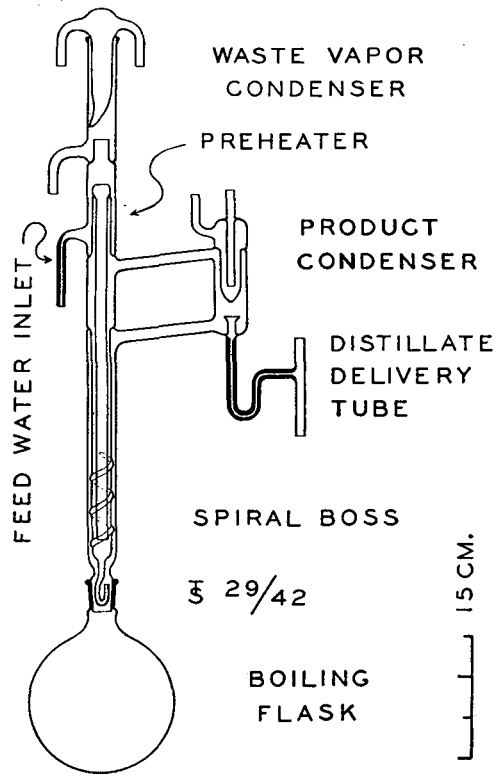

Figure 1. Distilling Apparatus

Receiver, feed water control unit, and connections for cooling water not shown

Under "$KMnO_4$ reduced" in Table I, the volumes given refer to the least amount of 0.002 *N* potassium permanganate solution required to produce the faintest visible pink color in 15 ml. of sample acidified with 2 drops of concentrated sulfuric acid and brought just to boiling. Probably these amounts are the minimum necessary to produce perceptible color.

To test for entrainment of liquid and solids from the boiling flask, 10 grams of sodium chloride were added to about 1000 ml. of water in the flask. To each 5 ml. of sample of distillate and of standard was added 0.2 ml. of acid silver nitrate reagent (5.097 grams of silver nitrate and 250 ml. of concentrated nitric acid per 1000 ml.). This was repeated with 0.1 ml. of 16% sodium acetate solution added to each sample. The resulting solutions or suspensions were compared in a Klett-Summerson photoelectric colorimeter using the yellow filter. Dial readings of the potentiometer in units are given in Table II.

2-Naphthol was used as a representative of a "phenolic" type of organic contaminant.

To each 10-ml. sample were added 4.5 ml. of diluted Folin-Ciocalteu phenol reagent (15 ml. diluted to 45 ml.) and 2.5 ml. of 20% sodium carbonate, and the reaction was allowed to go to maximum

color without turbidity at room temperature. Unknowns were compared with similarly treated standards containing 0.2, 0.5, 1, 2, 5, 10, 20, and 50 p.p.m. The "contaminant" was placed directly in the boiler or fed into the feed water intermittently as indicated in experiments 1, 2, and 3. Phenol itself was later introduced from a Mariotte bottle, substituted for the feed water control unit; the first 200 ml. of distillate were discarded, and the next 100 ml. saved for analysis. Phenol was determined in the same manner as naphthol, except that the reaction was conducted at 37.5° C. and unknowns were compared with standards containing 10 or 20 p.p.m. in the photoelectric colorimeter. Wire spirals were introduced into the degassing column, as indicated in Table II, but did not seem to improve performance appreciably, probably because the over-all effective exposure of water to steam was not much altered.

The loads of artificial contaminants imposed on the still were intentionally made abnormally heavy in order to obtain an idea of the manner of performance of functions anticipated in the design. There is evidence of removal of 2-naphthol from the vapor in the passage between the boiler and the product condenser. The decrease from that in the feed water to less than one fifth of this initial amount in the distillate may be considered satisfactory for many purposes. Although phenol itself is removed to some extent in the degassing tube, a substantial portion enters the boiler and part of this is redistilled into the product, in the ratio of about 7 in the distillate to 10 in the raw water. It is of little value that this is an absolute amount of only about 30% of that introduced. Because, as shown in the data for chlorides in Table II, entrainment is negligible, relatively large amounts of potassium permanganate may be added when minute amounts of phenol or other organic substances are likely to introduce significant errors in analytical work. This has been done in the preparation of water for the determination of iodine in blood, normally 6 to 8 micrograms per 100 ml. On the other hand, the error introduced by oxidizable organic matter when the distillation is conducted in the simple manner originally intended, using the present source of tap water, is too small to introduce appreciable error into the determination of 7 to 10 mg. of calcium in 100 ml. of blood serum by oxidation of the oxalate with potassium permanganate (Table I).

Principal Specifications of Apparatus

Outer shell, 35- to 36-mm. o.d. glass tubing
Vapor return tube, 25- to 26-mm. o.d.
Degassing tube, 18- to 19-mm. o.d., 480 mm. long
Feed water inlet, about 1-mm. bore, about 80 mm. long
Distillate delivery tube, about 1.5-mm. bore and about 100 mm. long below condenser, with short arm slightly less than half the length of long arm
Condenser (cold finger), 25- to 26-mm. o.d., 70 to 80 mm. long
Boiling flask, standard 2-liter flask with $ joint
Other dimensions may be estimated from Figure 1
With few exceptions, tubes are supported by fused joints at one end only, thereby avoiding difficulty due to unequal expansion during construction
Spiral boss shown in Figure 1 is tooled in vapor return tube and ground to fit closely but not tightly into outer shell

MODIFICATIONS TESTED OR SUGGESTED

An air lift for elevating raw water to the top of the degassing tube was designed to maintain an approximately constant level in the flask by recirculating water from the boiler or introducing fresh water as required. Its contribution to performance was doubtful, and it was abandoned in favor of the present simpler construction. An attempt was also made to control rate of feed of raw water by passing it through glass filter cloth at the top of the degassing tube. The rate of admission changed rapidly owing to deposition of lime scale. For even distribution of the film of water on the walls of the degassing tube, a smaller tube having a flange ground to give a clearance of about 0.3 mm. from the wall and centered by three minute projections has been suspended in the top of the degassing tube. Spirals of Chromel A or copper wire have also been used, and a spiral of 1-mm. glass rod is suggested.

A bare-wire immersion heater, suggested by those of Langdon (*5*), produced uniform boiling and facilitated satisfactory control of boiling rate. Its chief disadvantage was short life due to electrolytic action, probably resulting from unbalanced phases in the alternating current. An immersion heater in a silica shell was also tried, but in order to obtain adequate heating capacity, it had to be operated at a temperature rapidly destructive to the wire. An increase in surface area would have necessitated a unit of inconvenient size. For the present still, a conventional heater consisting of open elements of Chromel wire supported in a two-piece porcelain block, such as is used in Kjeldahl digestions, has been found satisfactory and surprisingly efficient; 450 to 500 watts are required in bare-wire immersion heaters and about 700 watts in the conventional outside heaters.

Carborundum chips have been used to promote smooth boiling over outside heaters. Three or four pieces of platinum foil, about 3 × 25 mm., with a spiral twist, have been suggested (*6*) for this purpose.

The cold finger has been made interchangeable by means of a standard-taper ground joint between it and the condenser chamber, to provide for substitution of cold fingers of varying capacity. It might advantageously be used also for cold fingers of other materials, such as silica, platinum, silver, tin, and tantalum. The present fixed capacity is between 3 and 4 liters in a day's run.

A condenser unit was tried in which the distillate dripped from the cold finger directly into a water-jacketed delivery tube. However, the unjacketed end inside the condenser unit required to keep out reflux from the walls, and necessarily wide enough to allow free passage of drops without contact, exposed more area of glass to hot vapor and possible drainage of condensate into the outlet than the entire delivery tube used at present. Data on conductivity were obtained on water collected through the water-cooled outlet; those on phenolic compounds, through the present delivery tube shown in Figure 1.

The effectiveness of the degassing tube might be increased by an increase in its length. The spiral wires assure uniform distribution of the film of incoming water, but do not otherwise improve performance.

A simple overflow outlet tube has been attached to the boiling flask to maintain a constant level in the flask, bleed off some of the water, and hold down the concentration of salts. The effect of the increase in rate of feed required was not tested. The chief objection to this device was greater fragility toward both thermal and mechanical shock. The plain standard flask has the advantage of ready availability for replacement, and accommodates enough volume to avoid excessive concentration in the boiler during a day's run. The bleeder would permit continuous operation over longer periods of time.

ACKNOWLEDGMENT

The author is indebted to Merle Siebert and Wayland Burgess, Department of Chemistry, University of Cincinnati, for measurements of the conductivity of the distillate; to Margaret Spies and Hettie Hughes, Christ Hospital Institute for Medical Research, for observations based on blood iodine determinations; and to G. F. Liebig for helpful criticism.

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Estimation of Methyl Bromide in Air

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THE widespread use of methyl bromide as a fumigant for the control of many insects has resulted in several analytical methods for its quantitative estimation. Most of these methods are based upon the hydrolysis of the methyl bromide with subsequent determination of the bromide ion. Typical procedures have been described by Glaser (*3*); Busby and Drake (*1*); Stenger, Shrader, and Beshgetoor (*6*); Chisholm and Koblitsky (*2*); and Lewis (*4*). However, none of these methods has satisfactorily filled the requirements for a rapid and convenient procedure for field work.

Actually, the delimiting field sampling requirements are that a sample be taken rapidly and accurately without the use of complicated apparatus. A gross method of this sort was suggested by Quayle (*5*) in work with hydrogen cyanide. This report deals mainly with an adaptation of this method for methyl bromide, wherein alcoholic potassium hydroxide is used as the absorbing and hydrolyzing medium.

EXPERIMENTAL PROCEDURE

A 2-liter sample of air containing methyl bromide was drawn by aspiration at controlled rates through two series-connected Fisher gas-absorbing scrubbers each containing 100 ml. of 5% alcoholic potassium hydroxide. After the solutions from the absorbers were combined, they were allowed to stand for 2 hours at room temperature to complete hydrolysis. The resulting potassium bromide was completely dissolved by the addition of 300 ml. of water and 150 ml. of 10% acetic acid solution, then titrated with standard 0.1 *N* silver nitrate solution with sodium eosin as the indicator.

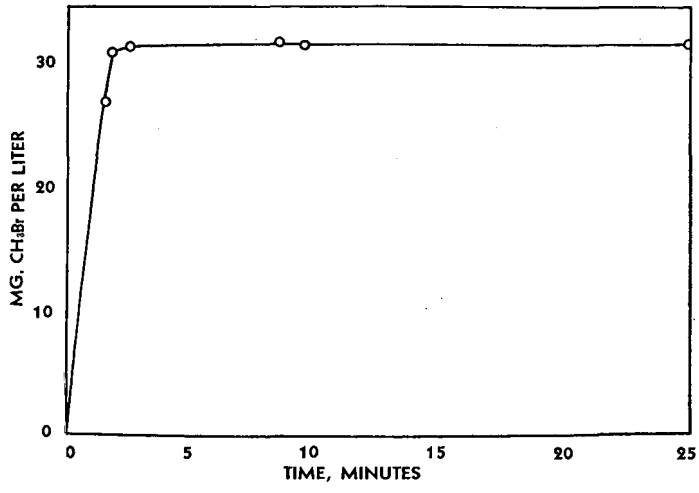

Figure 1

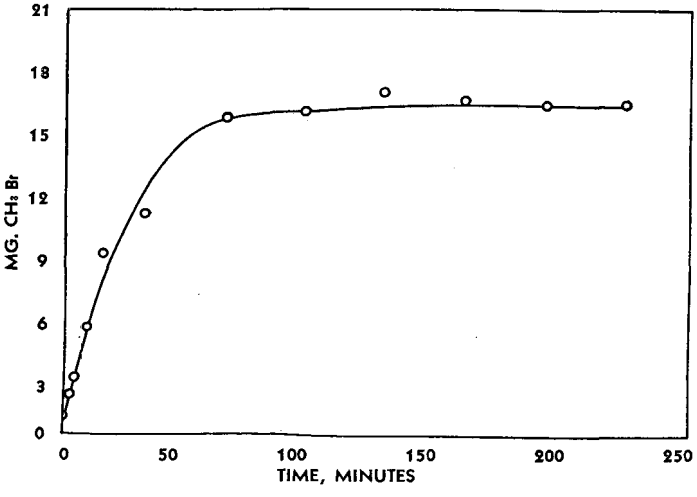

Figure 2

Table I. Absorption of Methyl Bromide at Various Rates

Rate of Aspiration, Minutes	Total Ml. of 0.1 *N* $AgNO_3$ to Titrate	Calcd. Mg. of Methyl Bromide per Liter of Air
1.50	5.80	27.2
1.75	6.70	31.4
2.50	6.75	31.6
6.00	6.85	32.1
7.00	6.80	31.9
29.30	6.80	31.9

DISCUSSION

Effect of Rate of Aspiration upon Absorption. The essentiality of a rapid sampling period required study of the effect of the rate of aspiration upon the completeness of absorption. This was done by varying the rate of aspiration, by means of capillary tubes inserted in the aspirating system, so that varying rates from 1.5 to 30 minutes were obtained. Two series-connected scrubbers were used for these runs. (The fumigator was charged with 48.9 mg. of methyl bromide per liter of air about 3 hours before sampling.) The results, graphically seen in Figure 1, show very clearly that a minimum of 2 minutes' aspiration will absorb all the methyl bromide under the conditions of this study. This was further demonstrated by using four scrubbers in the system, and taking each sample at the 2-minute rate. Pooled results so obtained were no different from those given by two scrubbers. It was shown, however, that one scrubber would absorb only 79% of the methyl bromide in parallel experiments. The third and fourth scrubbers were tested separately and gave no indication of the presence of even a trace of bromide.

Rate of Hydrolysis of Methyl Bromide in Alcoholic Potassium Hydroxide. The rate of hydrolysis of methyl bromide in alcoholic potassium hydroxide was determined by pipetting 10 ml. of 10% alcoholic potassium hydroxide solution and 10 ml. of alcoholic methyl bromide solution (approximately 1.6 grams per liter) into test tubes which were placed in a water bath at 25° C. and stoppered tightly with a waxed cork. The reactions were stopped at suitable intervals by pouring the contents of the tubes into 50 ml. of 2% acetic acid solution. The results, illustrated by Figure 2, indicate that a minimum of 2 hours was necessary for complete hydrolysis of the methyl bromide under the conditions utilized.

Rate of Dissipation of Methyl Bromide in 100-Cubic Foot Fumigator. When a known concentration of methyl bromide was introduced into a fumigator (100-cubic foot California vacuum fumigator for the Vacufume process, manufactured by the Union Tank and Pipe Co., Ltd.), the value determined was always lower than the theoretical. Therefore the variation of concentration of methyl bromide with time in the fumigator was evaluated. It was believed that the methyl bromide was either adsorbed on the walls of the fumigator, was reacting with the copper heaters therein, or both. A charge of 40 ml. of methyl

Table II. Rate of Hydrolysis of Methyl Bromide in 10% Alcoholic Potassium Hydroxide Solution

Reaction Time, Minutes	Total Ml. 0.1 *N* $AgNO_3$ to Titrate	Methyl Bromide, Mg.
0	0.04	0.9
2	0.24	2.2
4	0.30	2.8
8	0.57	5.3
16	0.96	9.0
32	1.16	10.9
64	1.68	15.7
96	1.70	15.9
128	1.80	16.9
160	1.75	16.4
192	1.76	16.5
224	1.75	16.4

Table III. Rate of Dissipation of Methyl Bromide in 100-Cubic Foot Fumigator

Sampling Time, Hours	Total Ml. of 0.1 *N* $AgNO_3$ to Titrate	Mg. of Methyl Bromide per Liter of Air
0	...	24.5
0.08	4.25	19.9
0.5	4.40	18.7
1	3.65	17.1
2	3.47	16.1
4	3.31	15.5
6	3.19	14.9

bromide (24.5 mg. per liter) was placed in the fumigator and circulated with an electric fan. Samples were withdrawn by the described method at 5 minutes, 30 minutes, and 1, 2, 4, and 6 hours. The results, shown in Figure 3, indicate that both adsorption and chemical reaction reduce the concentration of methyl bromide under these conditions; hence the seeming contradiction of Table I.

ACKNOWLEDGMENT

The authors wish to thank D. L. Lindgren for suggesting the problem and for certain materials and assistance.

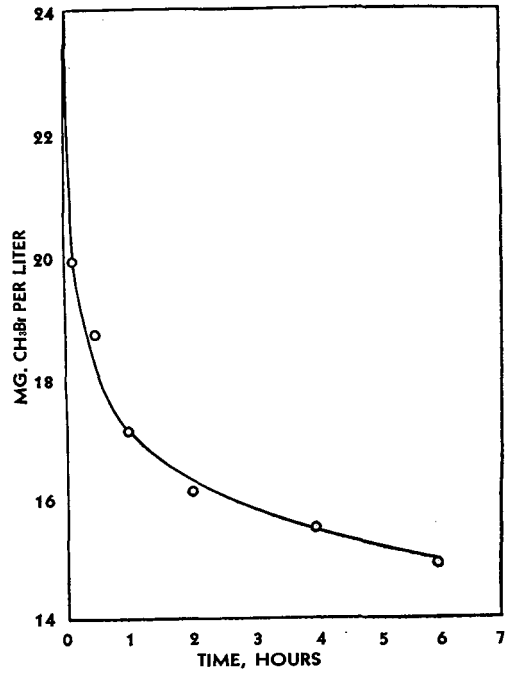

Figure 3

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Improvements in Microaeration Technique for Determination of Kjeldahl Nitrogen

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IN THIS laboratory difficulty was experienced in obtaining satisfactory nitrogen recoveries with the microaeration method of Sobel *et al.* (*2*). A study of the method has shown that it is reliable and practical if larger amounts of alkali are used and if the aeration is carried out at approximately 70° C. instead of room temperature as recommended by Sobel *et al.* In principle the latter is to be expected, because Dillingham (*1*) and others have stressed the importance of aeration temperature in analytical Kjeldahl nitrogen macro- and semimicromethods.

In order to aerate the Kjeldahl digest at an elevated temperature and trap the displaced ammonia in boric acid at a lower temperature, it is desirable to use a modified Sobel *et al.* microaeration tube. The authors have constructed tubes similar to those used by Sobel *et al.*, except that the side arm is at an angle of 45° with the inlet of the sealed-in bubbler of the microaeration tube (Figure 1). The tubes are assembled in zigzag formation in a rack with the digestion tubes inside the bath and the receiving tubes outside the bath. The rack can be easily constructed by making four 90° folds in a rectangular piece of 2-mesh hardcloth cloth, so that the ends of the original rectangle join at the base to clamp over the side of the bath. Short pieces of rubber tubing are used to complete the glass-to-glass connections between tubes, as in the Sobel method.

It was found that nitrogen could not be completely recovered from known amounts of ammonium sulfate or from protein-free blood plasma filtrates, even with prolonged aeration at room temperature or by increasing the aeration rate to the point where

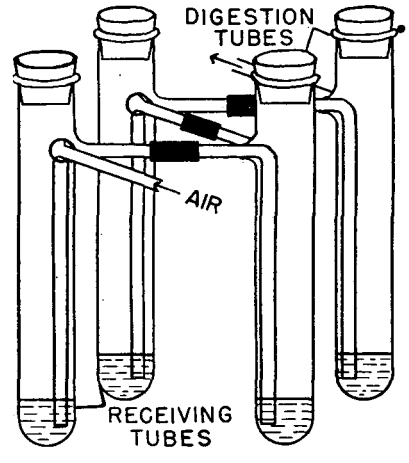

Figure 1. Setup of Aeration Tubes

Table I. Comparison of Sobel and Modified Methods in Determination of Blood Plasma Nonprotein Nitrogen

	Sobel Method		Modified Method	
Sample	Nitrogen found after aerating 30 min. at 25° C.	Additional N found after adding more alkali[a] and aerating 30 min. at 70° C.	Nitrogen found after aerating 30 min. at 70° C.	Additional N found after aerating 30 min. longer at 70° C.
	γ	γ	γ	γ
1	44.5	0	45.0	0
	39.7	3.9	44.5	0
2	147.2	1.9	149.5	0
	129.2	20.3	149.0	0
3	107.9	1.9	115.2	0
	106.9	10.2	116.6	0

[a] Following this, alkali concentration equaled that used in modified method.

loss by foaming was imminent. This was the case even though rapid aeration was continued for as long as 2 hours.

The effectiveness of raising the aeration temperature to 70° is indicated by the data in Table I. The samples used were protein-free plasma obtained from the blood of normal and adrenalectomized rabbits. Duplicate values agree well and the aeration is complete when the modified method is used. On the contrary, when the Sobel (*2*) method is used, the duplicate samples do not agree well and additional amounts of nitrogen are found if extra alkali is added and the aeration is continued for 30 minutes more at 70° C. The nitrogen recovered is approximately equal to the difference between the values obtained by the two methods. Various other data agree with the results in Table I.

MODIFIED PROCEDURE

The modified procedure for 10 to 200 micrograms of nitrogen is the same as described by Sobel *et al.* (*2, 3*) except that the aeration is carried out at 70° C.; the alkali is prepared by adding 19 ml. of saturated sodium thiosulfate solution to 481 ml. of a saturated solution of sodium hydroxide; to replace water lost by evaporation, thereby avoiding salt precipitation, each digestion tube is rinsed down with 1.4 ml. instead of 1.0 ml. of water; and 0.7 ml. of the alkali is added to each tube. Aeration is continued for 30 minutes at the rate of approximately 10 liters per hour.

ACKNOWLEDGMENT

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Determination of Tin in Manganese Bronzes

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IT IS stated in the literature that in the analysis of manganese bronzes, tin cannot be quantitatively precipitated as metastannic acid on account of the interference of iron (*2–4*). The authors have found that if a 5-gram sample is taken, a good excess of nitric acid is present, and the solution is boiled at least 0.5 hour, the interference of iron can be entirely overcome. When the solutions were not boiled but were allowed to stand on a steam bath at 95° C. for several hours in the usual manner, the recovery of tin was excellent for samples containing over 0.8% tin, but very poor (less than 45% recovery) for samples containing less than 0.5% tin. Inasmuch as over 95% of the manganese bronzes of commerce contain less than 0.5% tin, the importance of the method is readily seen. The procedure, which has now been in use in this laboratory for many months, has given astonishingly accurate and precise results even in the hands of routine operators.

The filtrate from the metastannic acid precipitate can be used for the determination of copper and lead by diluting to 200 ml., taking a 100-ml. aliquot, and electrolyzing the copper and lead. The lead dioxide deposit should be dissolved in nitric acid and the lead determined by the sulfate method. Determination of copper by the above procedures is especially advantageous for the analysis of copper in manganese bronzes containing less than 0.5% tin, for any unprecipitated tin would contaminate the copper deposit.

PROCEDURE

Dissolve a 5-gram sample in a covered 600-ml. beaker with 50 ml. of 1 to 1 nitric acid by heating on the steam bath. Boil for a minute or two on the hot plate to drive off the oxides of nitrogen, add 50 ml. of hot water and some paper pulp, and boil for 30 minutes. During the boiling keep the volume between 80 and 100 ml. by the addition of hot water. Filter the solution while hot through an 11-cm. No. 44 Whatman filter paper, containing some paper pulp. Swab the beaker and wash the precipitate well with hot 1% nitric acid solution.

Transfer the filter paper and precipitate to a 500-ml. Erlenmeyer flask and add 15 ml. of sulfuric acid, 5 ml. of perchloric acid (70%), and 10 ml. of nitric acid. Evaporate to strong fumes of sulfuric acid. Allow to cool, add 200 ml. of water and 75 ml. of hydrochloric acid, and reduce the tin with antimony trichloride and lead in the customary manner (*1*).

RESULTS

The results obtained by the authors on three representative manganese bronzes are shown in Table I.

Table I. Determination of Tin

Sample	Sn Present %	Sn Found %	Fe Present %
62b	0.97[a]	0.97 0.98 0.97	0.82
1	0.27[b]	0.27 0.27 0.26	1.32
2	0.10[b]	0.10 0.09 0.10	1.20

[a] National Bureau of Standards certified value.
[b] Average of three results by umpire A.S.T.M. method (*1*).

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RECEIVED July 7, 1948. The opinions expressed in this article are those of the authors and are not to be construed as representing the official views of the Navy Department.

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Simplified Countercurrent Distribution Apparatus

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COUNTERCURRENT distribution is an experimental method of great power and versatility in the analysis of mixtures of similar substances. Introduced only a few years ago by L. C. Craig of the Rockefeller Institute (4), it has already become one of the indispensable tools of the chemist.

The method consists in distributing a mixture in a certain way through a series of separatory funnels by means of a two-phase solvent system. The identity and quantity of the components of the mixture are determined from an analysis of the contents of each funnel after distribution.

One method for carrying out the distribution employs separatory funnels and hand shaking. This apparatus is readily available, and allows a wide choice of solvent volumes and quantities of material, but suffers from the disadvantage that a large number of separate operations is necessary. For a 24-plate separation, 288 shaking operations are necessary.

Another method currently in use employs a machine designed by Craig. This machine makes the necessary equilibrations and transfers automatically, but it is limited in solvent volume and is expensive.

A semiautomatic apparatus combining the best features of both these methods has now been developed. It is essentially a series of separatory funnels of special design arranged on a frame for shaking and semiautomatic transfer.

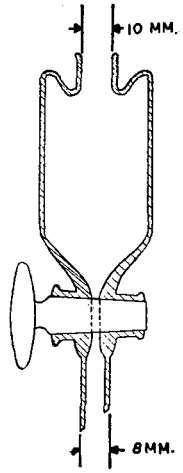

Figure 1. Countercurrent Distribution Plate

APPARATUS

Separatory Funnels. Each unit or "plate" consists of a separatory funnel with a re-entrant neck (Figure 1). These units can be made from standard Pyrex 60-ml. cylindrical separatory funnels by any competent glass worker. Steps in converting the funnels are:

Attach handles to each end of the funnel.

Heat and draw down a section beginning about 7.5 cm. from the stopcock and extending 1 cm. toward the neck, leaving a sharp shoulder and a short section 1 cm. in diameter and 2 cm. ong to form the neck.

After reheating the shoulder uniformly all around, push the neck into the body of the funnel.

Cut off the neck as short as possible and finish in the usual manner.

Cut off the tip of the funnel to a length of 2 cm.

A funnel made in this way will hold a total of 35 ml. A volume of 20 ml. can be shaken vertically in the funnel with no loss of contents. If greater volume capacity is desired, the diameter of the funnel rather than the height should be increased, because efficiency of mixing falls off as height is increased, and the number of plates the machine will hold also decreases. The standard funnel of the given dimensions occupies about 10 cm. (4 inches) of vertical height.

Shaking Frame. The funnels are attached to a vertical rod (by means of laboratory clamps) for the shaking and transfer operations. The rod is part of a frame (Figure 2) constructed of 0.5-inch aluminum rods (Fisher Scientific Co., Flexaframe) fastened together with standard connectors. The pivots are special connectors (Emil Greiner Co., No. G23150) whose setscrews are cut off about 0.125 inch to allow for rotation.

The apparatus is driven by an electric motor (Bodine Electric Co., No. NSE33R, $^1/_{10}$ hp. with speed-reducing gears attached in the ratio of 15 to 1) through a cam at a speed of 330 cycles a minute. The cam is a disk 1.5 inches in diameter set 0.25 inch off center. The total length of thrust is therefore 0.5 inch.

The cam actuates an aircraft valve tappet and guide assembly (Aircraft Engine and Parts Co., New York, N. Y.) which drives the shaking frame in vertical reciprocating motion. Power for the downward phase of the motion is derived from a spring between the upper pivot and the guide block. This spring supplies a force of about 40 pounds when compressed 0.5 inch.

Shaken together in this apparatus, 10 ml. each of butanol and water are brought into equilibrium within 2 minutes. A solute such as acetic acid, present in either phase, is distributed in the equilibrium concentration in both phases in the same length of time. The funnels are attached to the shaking frame, the tip of each funnel projecting into the neck of the one below.

PROCEDURE

At the start of a distribution the desired volume of the less dense solvent, saturated with the heavier solvent, is placed in each plate. The sample, dissolved in the desired volume of the heavier solvent, is then added to the topmost plate of the column, which is shaken until equilibrium is established. (An automatic timer controlling the motor is a great convenience.)

The lower layer of the first plate (plate 0) is now drained into plate 1 and a second volume of the heavier solvent is added to plate 0. (The added solvent does not contain an additional quantity of the sample.) This operation constitutes one transfer. After equilibrating, the lower layer of plate 1 is drained into plate 2, and that of plate 0 into plate 1, and fresh solvent is added to plate 1. This operation constitutes the second transfer. The addition of solvent is facilitated by an automatic pipet—e.g., Kimble No. 37075—at the top of the column.

The cycle of operations is repeated until the desired number of transfers is reached. If more than a single vertical series of plates is utilized, the transfer from the bottom of one series to the top of the next is made by detaching the funnel and carrying it up by hand.

RESULTS

Preliminary testing of the apparatus was carried out using butanol-water as the solvent system and acetic acid as the test substance. The time required to reach equilibrium was deter-

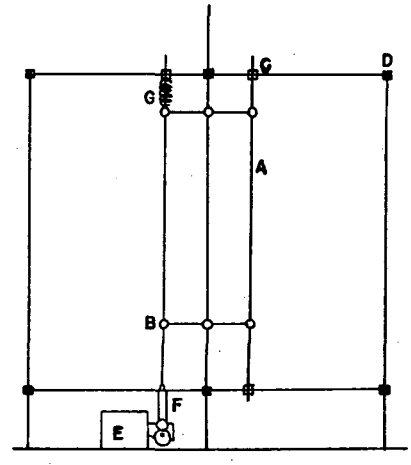

Figure 2. Shaking Frame

A. Moving rods
B. Pivots
C. Guide blocks
D. Standard connectors
E. Motor
F. Valve tappet assembly
G. Spring

Table I. Countercurrent Analysis of Lower Aliphatic Acids

Tube No.	Total Titration	Acetic Acid Calcd.	Propionic Acid Calcd.	Total Calcd.	Difference, Col. 2–Col. 5
0	0.28	0.00	0.33	0.33	0.05
1	1.41	0.04	1.38	1.42	0.01
2	2.79	0.20	2.65	2.85	0.06
3	3.77	0.63	3.14	3.77	0.00
4	4.15	1.53	2.58	4.11	0.04
5	4.44	2.75	1.56	4.31	0.13
6	4.36	3.73	0.71	4.44	0.08
7	4.17	3.92	0.25	4.17	0.00
8	3.60	3.19	0.07	3.26	0.34
9	2.71	2.11	0.01	2.12	0.59
10	1.25	0.99	0.00	0.99	0.26
Extra transfers	1.08	0.49	0.00	0.49	0.59
Totals	34.01	19.58	12.68	32.26	
%	100	57.6	37.2	94.8	

mined by a method similar to that of Barry (*2*) and was found to be 2 minutes for the given system. The K found for acetic acid was 1.23, in good agreement with the accepted value, 1.23 (*1*).

The efficiency of the distribution can be greatly improved for a given number of plates by carrying out a greater number of transfers than there are plates. The procedure is equivalent to procedure 2 of Craig (*3*, p. 523) except that most of the sample is retained in the apparatus, allowing calculation of distribution coefficients for all components.

The standard operations are carried through until all the plates of the apparatus are utilized. Additional transfers are then made. The sample from the last plate is not transferred to a new plate, but is immediately analyzed. The procedure is continued until the desired substance begins to show up in the last sample. Providing the K for the component is greater than 1, a distribution can be obtained equivalent to half again as many plates as are actually used.

In the analysis of a mixture of volatile aliphatic acids, obtained from another department of the college, an eleven-plate butanol-water system was used. After the fifteenth transfer the aqueous layer in the last plate had a titration value of 0.73 ml. of base, indicating the appearance of acid in this plate. The total contents of each plate were thereupon titrated, giving the values shown in column 2 of Table I.

Calculations. In the mathematical analysis of the system the following symbols are used:

r = serial number of a plate (numbering from 0)
T_r = quantity of any component in the combined phases of plate r
n = number of transfers completed
K = distribution coefficient, equal to 1.23 for acetic acid and 3.65 for propionic acid

The fundamental equation which governs the behavior of normal substances in the countercurrent system is

$$K = \frac{T_r}{T_{(r+1)}} \frac{(n - r)}{(r + 1)}$$

A derivation of this equation has been presented by Williamson (*5*).

Application of the fundamental equation shows that a maximum of acetic acid would appear in plate 7 and of propionic acid in plate 3. Preliminary values of T_r for propionic acid in the other plates were calculated from the total titration in plate 3, assuming this to represent pure propionic acid. The propionic acid value calculated for plate 7, subtracted from the total acid in that plate, gave a corrected value for acetic acid, from which column 3 of the table was calculated. New calculations from the corrected propionic acid in plate 3 now gave column 4. The sum of the calculated values for each plate is given in column 5. The difference (column 6) between this sum and the values found for each plate was within experimental error of titration over nearly all of the range. Of the total acidity of the sample taken, 94.8% was accounted for as acetic and propionic acids.

It is easily seen from the difference column that acetic and propionic acids quantitatively account for the results over tubes 0 to 7. Beginning with tube 8 there are positive deviations which are small but definitely larger than experimental error. These deviations indicate the presence of at least one additional acidic component, more soluble in water than in butanol.

ACKNOWLEDGMENT

The author's thanks are due to Beatrice Carrier Seegal for advice and encouragement, and to Herbert Wohl for technical assistance. This work was supported in part by a grant from the National Institute of Health.

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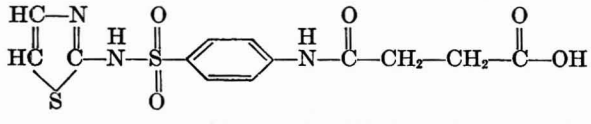
(2) Barry, G. T., Sato, Y., and Craig, L. C., *J. Biol. Chem.*, **174**, 209 (1948).
(3) Craig, L. C., *Ibid.*, **155**, 519 (1944).
(4) *Ibid.*, **161**, 321 (1945).
(5) Williamson, B., and Craig, L. C., *Ibid.*, **168**, 687 (1947).

RECEIVED November 10, 1948.

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

23. Sulfasuxidine (*p*-2-Thiazolylsulfamylsuccinanilic Acid)



Structural Formula of Sulfasuxidine

Sulfasuxidine exists in at least two polymorphic forms. Form I is stable below about 35° C. and melts at 125° C.; form II is stable above about 35° C. and melts at about 180° C. Crystals of either form may be prepared from methyl Cellosolve, 25% ethanol, or dioxane. Form I crystallizes if the solution is supercooled below 35° C. before crystallization; form II crystallizes at temperatures above 35° C. Good crystals of either form can be obtained by slow crystallization in the proper temperature range. Methyl Cellosolve was used to prepare form II and 25% ethanol to prepare form I in this study.

The commercial samples of sulfasuxidine are form I; form II in all cases transforms through the solid phase over a period of a few weeks to give I pseudomorphs of II.

SULFASUXIDINE I

CRYSTAL MORPHOLOGY (determined by W. C. McCrone).

Crystal System. Monoclinic.

Form and Habit. Rods elongated parallel to b from 25% alcohol. The most common forms and usually the only forms are the orthopinacoid {100}, clinopinacoid {010}, and hemiorthodome {101}.

Axial Ratio. $a:b:c = 5.095:1:3.793$.

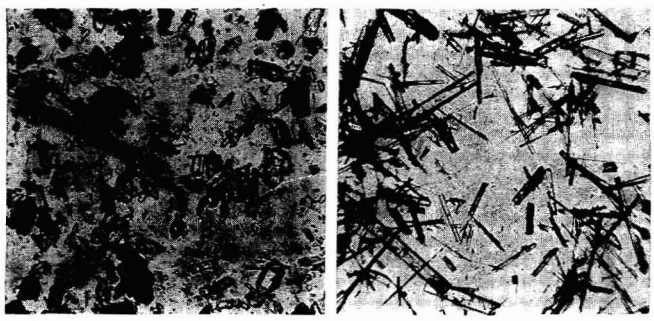


Figure 1. Sulfasuxidine

a. **Crystals of II (clear) and II pseudomorphs of I (opaque) from methyl Cellosolve**
b. **Crystals of I from 25% alcohol**

Interfacial Angles (Polar). 100 Λ $\bar{1}01$ = 116°.
Beta Angle. 127°.

X-Ray Diffraction Data (determined by W. C. McCrone and I. Corvin).
Cell Dimensions. a = 26.34 Å.; b = 5.17 Å.; c = 19.61 Å.
Formula Weights per Cell. 4.
Formula Weight. 341.
Density. 1.529 (flotation).

Principal Lines

d	I/I_1	d	I/I_1
16.39	0.56	4.57	0.96
12.79	0.15	4.34	0.23
9.67	0.13	4.19	0.13
8.62	1.00	4.04	0.34
8.08	0.23	3.87	0.19
7.17	0.07	3.74	0.30
6.48	0.19	3.07	0.20
6.03	0.75	3.16	0.50
4.89	0.45	3.26	0.13
4.70	0.07	3.52	0.87

Optical Properties (determined by W. C. McCrone).
Refractive Indexes (5893 Å.; 25° C.). α = 1.578 ± 0.002; β = 1.676 ± 0.002; γ = 1.710 ± 0.005.
Optic Axial Angles (5893 Å.; 25° C.). $2V$ = 58°.
Disperson. $v > r$, strong.
Optic Axial Plane. ⊥010; γ Λ c = 49° in obtuse β.
Sign of Double Refraction. Negative.
Acute Bisectrix. $\alpha = b$.
Extinction. β Λ c = 41° in acute β.
Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.653. R (calcd.) = 82.5. R (obsd.) = 81.8.

Fusion Data (determined by W. C. McCrone).
Sulfasuxidine decomposes slightly on melting and supercools to a glass. Some very slow crystallization is observed if the melt is seeded and held at a temperature just below the melting point.

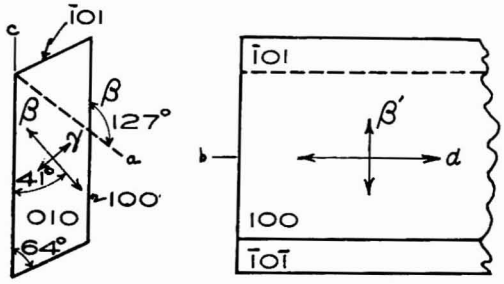


Figure 2. Orthographic Projection of Typical Crystal of Sulfasuxidine I

Commercial samples of sulfasuxidine I melt at 125° C. and resolidify as form II, which then melts on continued heating at about 180° C.

SULFASUXIDINE II

Crystal Morphology (determined by W. C. McCrone and Ralph Johnson).
Crystal System. Triclinic.
Form and Habit. Massive crystals from methyl Cellosolve.

X-Ray Diffraction Data (determined by I. Corvin).

Principal Lines

d	I/I_1	d	I/I_1
13.17	0.22	4.24	0.28
6.40	0.30	4.02	0.30
5.43	0.30	3.89	0.38
4.58	0.40	3.75	1.00
4.43	0.37	3.55	0.22
4.30	0.33	3.42	0.44

Optical Properties (determined by Ralph Johnson).
Refractive Indexes (5893 Å.; 25° C.). α = about 1.54; β = about 1.61; γ = 1.73.
Optic Axial Angles (5893 Å.; 25° C.). $2V$ = about 80°.
Dispersion. None.
Sign of Double Refraction. Positive.

Book Reviews

Germ-Free Life Studies. Lobund Report No. 2. *James A. Reyniers*, ed. vi + 162 pages. University of Notre Dame, Notre Dame, Ind., 1949. Price, $2.50 clothbound, $1.75 paperbound.

This report, the second of a series prepared by the Laboratories of Bacteriology, University of Notre Dame (Lobund), and devoted to germ-free life studies, consists of three closely related articles; the first two describe the rearing of germ-free chickens and some observations on germ-free bantam chickens, while the third outlines briefly the present status of terminology in germ-free life studies and advances several suggestions for further consideration.

The work carried out at Lobund since 1928 has resulted in the development of techniques for the routine production and maintenance of germ-free experimental animals both on a simple, short-term (30-day) basis and on a more elaborate, more flexible, longer term basis (the Reyniers germ-free system); these procedures, particularly as applied to the chicken, are well outlined in the text and are illustrated by an excellent series of schematic diagrams and photographs. The discussions of egg-surface disinfecting agents, emphasizing considerations of surface sterility and embryo viability, are good. A substantial beginning has been made toward a thoroughgoing description of the germ-free chicken as a life form.

The typography and binding are good; the reviewer feels this report is of interest to biological chemists and biologists in general.

Albert P. Doerschuk

Chemistry of Specific, Selective, and Sensitive Reactions. *Fritz Feigl. Ralph E. Oesper*, translator. xiv + 740 pages. Academic Press, Inc., 125 East 23rd St., New York 10, N. Y., 1949. Price, $13.50.

Ninety years ago, H. Schiff used paper impregnated with silver carbonate for detecting uric acid in a drop of urine and two years later C. F. Schönbein published some studies on tests made on filter paper, which inspired Fr. Goppelsroeder (1861–1907) whose "Kapillaranalyse" was published in 1910. Eight years later Feigl began to publish papers on spot tests, or drop reactions, and soon became the most active worker in this field. Since the announcement of the ionization theory by Arrhenius in 1887, this has become the most remarkable and most important development in the field of analytical chemistry. In this new book by Feigl, ably translated by Oesper, more than 1600 papers published during the past 25 years are reviewed in a masterly manner. Of these numerous papers, at least 182 were written by Feigl with his many students. Next comes I. M. Kolthoff with 36 papers; in the opinion of the reviewer, Kolthoff is now our most distinguished chemist in the field of chemical analysis.

The book is interesting and most readers will learn something on every page. However, they should have a very good idea of organic chemistry and cognizance of the Werner valence theory, at least to the extent to which it has been adopted in Fritz Ephraim's "Inorganic Chemistry." Most readers will find Chapter VI—the effect of atomic groupings on the specific and selective activity of compounds in inorganic analysis—the hardest to read. It covers 215 pages (nearly one third of the entire text) and gives the graphic formulas of more than 400 organic compounds that can be used to advantage in analytical chemistry as reagents; they are often more specific, more selective, and more sensitive than inorganic reagents.

On first examining the book, one is inclined to think the style a bit pedantic. However, after reading a few pages, the reader becomes so interested in the content that he forgets this because, after all, Oesper has expressed himself clearly and shown that he has understood as well as translated Feigl's book.

The sentence on page 64 that reads, "It is therefore expedient to treat the experimentally measurable total result of an adsorption as a complex compound that cannot be stoichiometrically defined," requires a second reading to make its meaning clear.

The term "indubitable tests" (page 19) will not be misunderstood, although in common conversation the word "reliable" might be used. The Latin phrase *in statu nascendi* used on page 477 and elsewhere, the French word *milieu* on page 596, and the German word *Reinnickel* on page 660 merely show the erudition of the writer and do not help the reader.

The phrase on page 446, "colloidality is the precursor of true solution," is terse, but the common use of "elucidate" instead of "explain" and "demonstrate" instead of "prove" is a fault common to many scientific writers.

The "chemical assailability by dissolved reagents" on page 412 is not hard to understand and the use of the word "measurability" instead of the more common "mensurability" does not displease the reviewer.

On page 7 "1 mm.^{3}" is used twice and "0.001 ml." only once. The lack of consistency is more serious when on page 445 we find the millimicron written $\mu\mu$ while on page 671 it is written mμ, a preferable form. It is true that $\mu\mu$ was used by Wo. Ostwald in his "Applied Colloid Chemistry" (1917) and is in the 1910 edition of "The Encyclopaedia Britannica," but today it is not used by careful writers, because it apparently denotes one millionth of a micron and not one thousandth.

Throughout the book it was a pleasure to find words derived from Latin roots preceded by Latin prefixes but it is not clear why Oesper prefers "divalent" to "bivalent" or "niobium" to "columbium." In England and on the continent, the element is named after the beautiful daughter of Tantalus and Dione in Greek mythology, but atomic weight tables published in the United States contain no mention of niobium.

Names such as "peroxoorthotitanic" acid (page 80) are a little awkward, "fuchsin" is better than "fuchsine (pages 98 and 99), and the description of Fehling's solution as "an alkaline tartrated cupric solution" (page 98) is somewhat objectionable.

The formulas for sodium thiostannate on page 36 and for starch on page 561 are incorrect, and the reviewer does not like the term "metal acids" (page 559).

Little attention is paid to mathematics and the explanations of reactions are mostly on a purely qualitative basis. In a table on page 428 it is stated that alkaline earth sulfates and borates are insoluble in water and in acids. This is a pretty strong statement, for 0.22 gram of $CaSO_4.2H_2O$ and 0.40 gram of $Ca(BO_2)_2.2H_2O$ will dissolve in 100 ml. of water; both these salts are considerably more soluble in dilute mineral acids and barium sulfate dissolves in concentrated sulfuric acid.

On page 471 it is stated, "The familiar reaction $HgCl_2 + SnCl_2 = SnCl_4 + Hg^\circ$ . . . . . gives a precipitate which consists entirely of free mercury." Every sophomore knows that mercurous chloride is first formed in this reaction and some of it remains unless excess stannous chloride is added. The formula for "cellulose sulfuric ester" is given as $C_6H_7O_5(SO_3K)_3$ on page 481. Strictly speaking, this is not the ester but its potassium salt and the molecule is probably considerably larger than corresponds to this formula.

The statement on page 514 that "ammonium permutite can be kjeldahlized" may not be intelligible to all readers. Some may not know what Lloyd's reagent is (page 518) or what is meant by Dalton compounds (page 548) or "formula-pure stannic phosphate" (page 550), but they learn (page 547) that a Dalton compound is a "stoichiometrically defined compound."

The explanation on page 661 of the varying behavior of calcium sulfate in the minerals calcite and aragonite is a bit far-fetched. When samples of each mineral are "spotted" with a solution containing manganese and silver ions the aragonite reacts almost immediately to give a black spot containing manganese dioxide and finely divided silver. The reaction is probably:

$$Mn^{++} + 2Ag^+ + 2\,CaCO_3 = MnO_2 + 2Ag^\circ + 2Ca^{++} + 2CO_2$$

which is not quite the same as the sum of the reactions that Feigl assumes. On page 662 the fact that a sample of calcite reacts with a solution containing sulfate and a saturated, aqueous solution of nickel dimethylglyoximate at room temperature and does not give the test in a hot solution is attributed to lower solubility of calcium sulfate in hot water than in cold. A handbook at my desk states that 0.18 gram of calcium sulfate dissolves in 100 ml. of water at 0° and 0.16 gram at 100°; this 11% difference is not very large when the reaction takes place in 0.05 ml. of solution. It is true that Poggiale and Mariagnac found the maximum solubility of calcium sulfate at approximately 35°.

The reviewer does not like the title of Chapter XI (page 613), "Genetic Formation of Materials and Topochemical Reactions." By "materials" is meant the chemical compounds formed in spot tests.

The explanation of the reductions caused by heating solids with ammonium iodide is ingenious. It is assumed that ammonia and hydriodic acid are first formed by thermal dissociation and then the ammonia breaks down into nitrogen and 6 atoms of nascent hydrogen which is largely responsible for the reducing effect. As hydriodic acid is a fairly strong reducing agent, it seems unnecessary to assume the formation of "active hydrogen."

Feigl has done a beautiful job in critically examining the literature of the past 25 years and Oesper is to be lauded rather than criticized for his painstaking translation. The book is well printed, contains remarkably few misprints, and is well worth the price charged.

WILLIAM T. HALL

The Analyst's Calendar

Optical Society of America. Hotel Statler, Buffalo, N. Y., October 27 to 29

Conference on X-Ray and Electron Diffraction. Mellon Institute, Pittsburgh, Pa., November 7 and 8

American Council of Commercial Laboratories. Miami, Fla., December 5 to 7

Third Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 30 to February 2, 1950

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. William Penn Hotel, Pittsburgh, Pa., February 15 to 17, 1950

Third Annual Summer Symposium. Ohio State University, Columbus, Ohio, June 16 to 17, 1950

AIDS FOR THE ANALYST . . . . .

Agitator for Mattson Electrodialysis Cell. W. H. Slabaugh, The State College of Washington, Pullman, Wash.

IN using the modified Mattson cell (as supplied by the American Instrument Company) for the electrodialysis of a suspension of clay material, the ordinary type of paddle or blade stirrer was found unsatisfactory. The shape of the cell, which is narrow in comparison to its width and depth (1.8 × 10 × 17 cm.), required a stirrer of special design. Because of the necessity of obtaining uniform dispersion during electrodialysis, the agitator shown was developed.

The principle of this agitator involves the transfer of the circular motion of an ordinary stirring motor to a reciprocal motion by means of an eccentric. At the same time, compressed air is allowed to escape from the bottom end of the stirrer, so that a bubbling action is combined with the mechanical effect. As a result of these two actions, the suspension in the cell remained uniformly dispersed throughout the period of electrodialysis, which often extended to 9 hours.

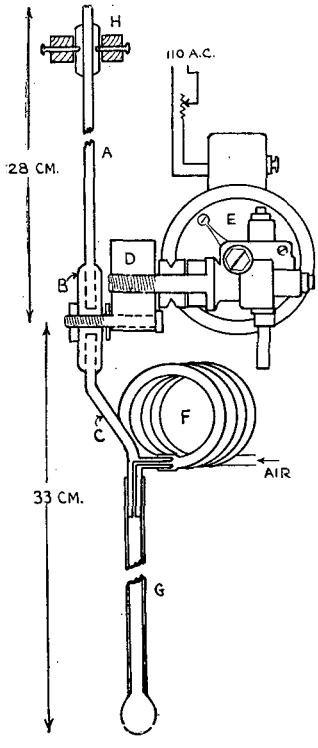


Figure 1

Figure 1 shows the stirrer in a front view. The assembly, *A*, *B*, *C*, was mounted eccentrically on the hub, *D*, which was threaded at its center to fit the lower speed drive shaft of a Fisher No. 14-498 stirring motor, *E*. *A* and *C* were made of 3/16-inch brass rod, *B* was made of 1/2-inch brass rod, and the hub was made of 2-inch brass rod. The lower portion of *C* was drilled and a side arm soldered thereto, so that air could be piped into the stirrer. Air was supplied to this side arm through a rubber tube, inside which was a suitable wire spiral, *F*. The lower end of the stirrer, *G*, was made of glass tubing with a bulb on the end in which were two small holes. The glass tube was attached to *C* by means of a thermoplastic cement. The pivot, *H*, was made of brass rod which was drilled to accommodate the upper end of *A*. A variable resistance was placed in the 110-volt circuit to the motor in order to control the speed of the stirrer.

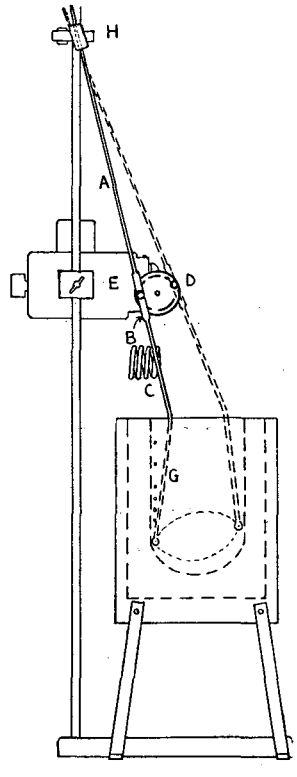


Figure 2

The brass hub may be replaced with bar stock, but it was found that the heavier type of hub resulted in greater stability at higher speeds. The whole assemblage mounted on an ordinary ring-stand was free of undesirable vibration at speeds up to 200 r.p.m.

Figure 2 indicates a side view of the agitator and its action when used with the electrodialysis cell.

Use of Interference Filters in the Reduction of Error Due to Calcium in the Flame Photometric Determination of Sodium. P. F. Pratt and W. E. Larson, Iowa State College, Ames, Iowa.

IN the determination of sodium, using the Model 18 Perkin-Elmer flame photometer, calcium has been found to give a positive error. In an effort to reduce this error a Baird interference filter was substituted for the Corning filters which are standard equipment for the photometer. The interference filter has a maximum transmission between 5790 and 5990 Å. and is used with an auxiliary glass filter to remove unwanted blue transmission bands.

Table I. Calcium Error Found for Sodium Determination with Baird Interference Filter and Corning Filters

		Interference Filter		Corning Filters	
Na	Ca	Na found	Error	Na found	Error
P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
0	100	0.3	0.3	0.5	0.5
0	200	0.8	0.8	1.7	1.7
0	400	1.7	1.7	3.1	3.1
0	600	2.5	2.5	4.8	4.8
0	800	3.3	3.3	6.7	6.7
0	1000	4.2	4.2	8.2	8.2
20	100	20.5	0.5	21.1	1.1
20	200	21.1	1.1	22.2	2.2
20	400	22.2	2.2	24.6	4.6
20	600	23.7	3.7	26.7	6.7
20	800	24.5	4.5	28.8	8.8
20	1000	25.6	5.6	31.0	11.0

The test solutions were 2 *N* in ammonium acetate and 0.2 *N* in magnesium acetate [Attoe, O. J., and Truog, E., *Soil Sci. Soc. Am. Proc.*, 11, 221–6 (1946)]. Sodium was added as sodium chloride and calcium as calcium acetate.

Table I gives the calcium error for both the interference filter and the Corning filters. The error, using the interference filter, is for all cases about half that using the Corning filters. Expressed as instrument readings, the error was the same at 0 as at 20 p.p.m. of sodium. However, as a result of a nonlinear relationship between the instrument reading and the sodium concentration, the error, expressed as parts per million of sodium, was greater at the higher level of sodium.

In the flame photometric estimation of sodium, corrections for the amount of calcium are smaller when the interference filter is used. Thus, any errors in calcium determinations make smaller errors in the corrections for calcium. These corrections can be made conveniently from a graph, in which the error, expressed as photometer readings, is plotted against the calcium concentration.