ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

A Progress Report

THE Section of Analytical Chemistry of the International Union of Pure and Applied Chemistry, organized at the meeting of the union last September in Amsterdam, has just announced its membership.

The president is C. J. Van Nieuwenburg of the Delft Technical Institute, Netherlands; the European secretary is Paul E. Wenger of l' Ecole de Chimie de l'Université, Geneva, Switzerland, and the American secretary is Samuel E. Q. Ashley of the General Electric Company, Pittsfield, Mass. The vice president is I. M. Kolthoff of the University of Minnesota, long associated with the activities of the International Union.

Other members of the section as reported to this publication are: Fritz Feigl of the Laboratorio da Produção Mineral, Rio de Janeiro, Brazil; Edward Wichers of the National Bureau of Standards, Washington, D. C.; Jan Gillis of the University of Ghent, Belgium; Shanti S. Bhatnagar of the University Chemical Laboratories, Lahore, India; R. C. Chirnside of General Electric Co., Ltd., Wembley, England; F. E. Beamish, University of Toronto, Canada; N. Strafford of Imperial Chemical Industries, Ltd., Manchester, England; and G. Charlot of l'Ecole de Physique et de Chimie, Paris, France.

This membership will function until the union again meets, this time in the fall of 1951 in New York City.

At the present time the section membership is concerned with the establishment of two new commissions, one on physicochemical constants and the other on the expression of analytical results. The Commission on New Analytical Reagents and Reactions, which has functioned for many years and has provided the most direct contact for analysts with International Union activities, will continue under the sponsorship of the Section of Analytical Chemistry.

Despite the fact that the membership of the section as at present constituted is considered to be temporary, the international reputation of the members and their long-sustained interest in union affairs lead us to believe that the section will maintain a most active status between now and the 1951 meeting in New York.

The new policy of the International Union of Pure and Applied Chemistry of establishing sections is a vast improvement over the former organizational structure. So far, Sections on Inorganic Chemistry, Organic Chemistry, Physical Chemistry, Analytical Chemistry, Biochemistry, and Applied Chemistry have been established. Under the new organizational structure, much broader approaches can be made to scientific questions of international interest than was possible under the more restricted and narrow commissions setup.

It has been felt by analysts interested in the International Union that the Commission on New Analytical Reagents and Reactions failed to provide an opportunity to discuss many problems pressing for an international agreement. With the organizational machinery now available, more rapid progress can be made.

The editors of this publication will continue to keep its readers informed on all future developments of the Section of Analytical Chemistry.

The American secretary will be delighted to receive suggestions from American analytical chemists interested in furthering the progress of the Section of Analytical Chemistry of the International Union.

Analysts and Public Safety

ANALYTICAL chemists are guardians of public health, yet this aspect of their contribution to society is rarely indicated. How to bring about such recognition on the part of the public constitutes a challenge to the profession that should no longer be ignored.

Several possible courses of action should be considered. First and foremost, there is a crying need for some highly qualified analyst with broad experience and a flair for popular style writing to write a book which will humanize the analyst and, at the same time, portray the important role he plays in protecting the public's health and general well-being.

Food, shelter, and clothing are but a few of the prime essentials of life requiring in one form or another the stamp of approval of the analyst. The services of the medical profession will be weakened if the quality of the products used by physicians, surgeons, and dentists is not maintained at the highest possible levels.

Perhaps the time has arrived when we should sponsor a series of symposia for each of the more important fields where quality control touches directly the health and safety of the American public. In certain activities, as, for example, atmospheric contamination, considerable original research work must be done in developing quick and accurate analytical methods. It will be very difficult to solve many of the problems of air pollution until analysts can provide considerable necessary fundamental information about the form and concentration of the products causing the contamination of the atmosphere in many industrial areas.

HIGH-FREQUENCY TITRATIONS

A Study of Instruments

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High-frequency titrimeters that measure frequency changes during the course of a titration are described. These instruments are stable, sensitive, easy and convenient to use, and suitable for differential titrations. Titration curves for acid-base, precipitation, soluble complex, and oxidation-reduction reactions are presented. A mechanism to explain observed frequency changes is discussed.

IN ANY high-frequency titration, the titration vessel containing the solution is placed in the field of a high-frequency oscillator. Then, depending on the method of loading this oscillator with the vessel and the type of circuit used, there are changes in plate or grid currents or voltages, or in frequency as the titration proceeds, due to changes in composition of the solution. A plot of any one of these quantities against volume of standard solution gives a curve with a break in it at the end point of the titration, much like a conductometric titration curve.

To perceive the end point in a high-frequency titration, electrodes, indicators, and physical contact of any sort with the solution are not required. There are at present many quantitative chemical reactions which are unsatisfactory for use in titrations because there exist neither good color indicators nor electrodes for potentiometric titrations, or because conventional conductance electrodes become fouled during the titration. Such reactions might be suitable for high-frequency titration if there is a sufficient difference in loading between reagents and products.

Despite the numerous possibilities for new methods of analysis with high-frequency titration, no highly adequate instrument has yet been described in the literature. All instruments previously described are defective with respect to at least one of the following requisites of a good analytical instrument: sensitivity, stability, and speed and ease in operation.

In this paper, a high-frequency titrimeter is described which gives promise of developing into a good analytical instrument. A mechanism for the observed loading effect is discussed. Titration curves with good and accurate end points are given for many different reactions.

PAST WORK ON HIGH-FREQUENCY TITRATIONS

The earliest detailed account of the method of high-frequency titration and its application to various titrations was excellently given by Jensen and Parrack (*10*). A tuned plate–tuned grid oscillator with the titration vessel inserted in the coil of the plate circuit was employed. As the titration proceeded, changes in plate current were read from a metering system. This work was reinvestigated in this laboratory. The instrument was found to be unstable, as it was extremely sensitive to body capacity and to temperature variations in the oscillator tube. At adequate sensitivity, it was prone to go out of oscillation. Several modifications were made to stabilize this type of circuit. Although some improvement was obtained, it was apparent that the characteristics of the tuned plate–tuned grid circuit rendered good stability and high sensitivity incompatible (*12*).

Blake has been working in the field since 1945 or earlier, and has described several types of titrimeters (*4*). One of the most recent was a differential arrangement. Two condenserlike titration vessels were used, both fed by the same oscillator. The voltage across each vessel was rectified and the difference measured. Titration took place in only one vessel, the other being filled with a sample of solution whose composition remained constant. Diehl and co-workers (*3*) loaded a condenser-type circuit element with the titration vessel and measured a voltage change as the titration proceeded. Recently, the tuned plate–tuned grid oscillator circuit was modified to measure changes in grid current rather than plate current (*2*). None of these instruments was reinvestigated in this laboratory. However, a study of these articles did not change the conclusion reached after studying the Jensen and Parrack instrument—namely, that there are unexplored certain aspects of instrumentation which need further investigation before the high-frequency titration method may be widely applied in analytical chemistry.

Since this article was submitted for publication, there have appeared two more papers of significance on high-frequency titrations. Anderson *et al.* (*1*) describe a titrimeter which measures grid current and seems superior to the instruments previously mentioned. West *et al.* (*19*) describe a heterodyne titrimeter operating at 4 Mc. per second, in which the titration vessel loads a coil type of circuit element.

MECHANISM OF LOADING EFFECT

The plate or grid currents or voltages of the oscillator circuit change as the composition of the solution loading the oscillator circuit changes during a high-frequency titration. This effect is not clearly explained by past work. As the work described in this paper proceeded, a probable mechanism for the observed effects developed. In this section, the chronological order of the work which led to acceptance of this mechanism is outlined, together with the mechanism itself. Confirmatory data are given in following sections.

During a study of the modified Jensen and Parrack titrimeter (*12*), it was observed that the sensitivity dropped off below and above certain optimum concentration regions. Within this region, the sensitivity was adequate for carrying out titrations; outside this region, the plate current became insensitive to changes in composition. This would not have been the case had the loading been of a purely resistive nature. In fact, two pieces of evidence indicated that the loading was predominantly capacitative.

1. When a tuned plate–tuned grid oscillator operates on the steep portion of its tuning curve, it is extremely sensitive to small changes in capacity—i.e., small changes in capacity give large changes in plate current. The Jensen and Parrack titrimeter is stated to have greater sensitivity the more vertical the steep portion of the tuning curve becomes. This would be a direct and necessary consequence if the loading were capacitative (*10*).
2. When a high-frequency field is applied to an electrolytic solution there are an absorption of energy and a change in dielectric constant of the solution, as predicted by Debye and Falkenhagen (*8*) and as shown experimentally by several workers (*5, 9, 16*). The absorption of energy and the dielectric constant are also functions of the concentration at a particular frequency.

When the electrolytic solution loads many types of high-frequency oscillators, the change in dielectric constant causes a change of frequency of the oscillator. The change of frequency of an oscillator due to the change in dielectric constant of an electrolytic solution is shown in Figure 1 as a function of concentration at a particular frequency. The curve of energy absorption *vs.* concentration at the same frequency is also given in Figure 1. These curves are a semiquantitative reproduction of data from the work of Forman and Crisp (*9*). The curve of frequency *vs.* concentration of these authors is identical in form to that observed with all high-frequency titrimeters studied by the present authors (see Figure 10). It therefore appears that the titrimeter response is related to the change of dielectric constant with concentration when a particular high frequency is applied to the solution in the titration vessel.

The frequency *vs.* concentration curve of Figure 1 shows why the titrimeter response drops off above and below a certain concentration region. A response can be obtained only in that concentration region where the dielectric constant of the solution (and oscillator frequency) change with concentration. In the following discussion, the concentration range included by the steep portion of the frequency *vs.* concentration curve is defined as the "region of maximum sensitivity" of the titrimeter, because it is here that given changes in concentration produce the largest changes in dielectric constant of the solution and frequency of the oscillator. The mid-point of this region is defined as the "concentration of maximum sensitivity" of the titrimeter.

The data of Forman and Crisp allow a prediction of the concentration of maximum sensitivity for various electrolytes. These authors (*9*) have given a simple empirical relation between the concentration of a solution and the frequency at which the absorption of energy is a maximum: $\lambda c = K$, where λ is the wave length in centimeters, c is the normality, and K is a constant characteristic of the electrolyte considered. Figure 1 shows that the mid-point of the steep part of the frequency *vs.* concentration curve corresponds closely to the maximum in the energy absorption *vs.* concentration curve. This mid-point also gives the concentration of maximum sensitivity, as defined above. Therefore, the concentration of maximum energy absorption, as calculated using the formula of Forman and Crisp, should closely correspond to, and be a measure of, the concentration of maximum sensitivity of the titrimeter.

The observed concentrations of maximum sensitivity for the modified Jensen and Parrack titrimeter, and for several other titrimeters operating at greatly different frequencies, agree well with those calculated for maximum absorption using the above empirical relation. This agreement is taken to mean that the loading of the oscillator by the solution is predominantly capacitative in all the titrimeters studied, and that the observed changes in plate or grid currents or voltages, or in frequency, are related more fundamentally to changes in dielectric constant of the solution than to resistance.

This explanation of the loading effect is given in considerable detail, even though its elucidation is not a primary purpose of the investigation. The reason for this is threefold: First, there has been a tendency among most workers to assume that high-frequency titration is dependent upon the same properties of the solution as conductometric titration, and that the response or loading effect is a direct function of the conductance (or resistance) of the solution. Actually, as shown above, the response is more fundamentally and simply related to the dielectric constant. Secondly, this mechanism explains simply the dependence of the sensitivity of the titrimeter upon concentration and frequency. Thirdly, this mechanism is the basis upon which the instruments described in the following sections are designed. In so far as these instruments operate successfully, they indirectly justify the reasoning by which they were developed.

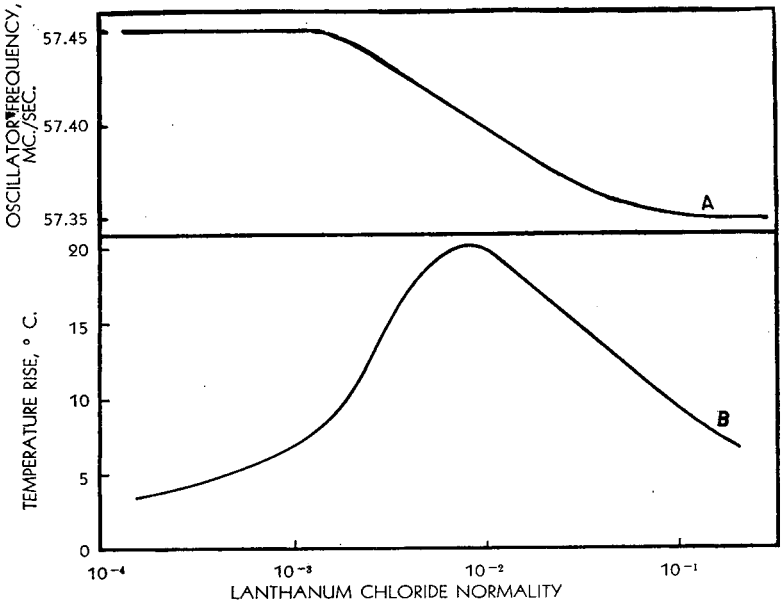

Figure 1. Dependence of Energy Absorption and Dielectric Constant of Solutions of Lanthanum Chloride upon Concentration

At 57.45 Mc. per sec. (from Forman and Crisp, *9*)
A. **Dispersion of dielectric constant measured by change in oscillator frequency**
B. **Calorimetrically measured energy absorption**

FREQUENCY-MEASURING TITRIMETER

Assuming that the loading of the oscillator by the titration vessel was capacitative, and that the above loading mechanism was correct, it was reasoned that a frequency change should be observed in the course of a titration. Because oscillators may be stabilized more easily and simply with respect to frequency than with respect to output current or voltage, it was felt that an instrument measuring frequency changes during a titration might come close to satisfying the requisites of a good analytical instrument. This proved to be the case.

Four frequency-measuring titrimeters were built. The first operated at about 5 Mc. per second, using the simple Clapp oscillator circuit (*6*). The work done with this instrument was largely exploratory in nature. Although its maximum sensitivity fell in a concentration range too dilute for much practical use, the data obtained confirmed belief in the mechanism for the loading effect and paved the way for improved instruments.

A second titrimeter was built using the same oscillator circuit as the first, but operating at a higher frequency of 30 Mc. per second. The maximum sensitivity for this instrument still fell in a rather low concentration range, but many satisfactory titrations were performed and titration curves with good end points were obtained.

It was attempted to extend the Clapp circuit to 100 Mc. per second, but this proved unsuccessful. A third instrument was built according to a conventional tuned plate circuit (*13*) at 100 Mc. per second. Although it could be used in titrations, it was not sufficiently stable as a good analytical instrument.

A fourth instrument, operating at 360 Mc. per second, has shown remarkable stability. This instrument employs a quarter-wave-length concentric line. Fragmentary study of its properties shows its maximum sensitivity to occur in a concentration range high enough to be practical for most analytical titrations. This work is still under way.

It is the description of these instruments—their construction, properties, use, and data obtained with them—that forms the body of this investigation.

PRELIMINARY STUDY AT 5 Mc.

Description of 5-Mc. Titrimeter. In building a high-frequency titrimeter which changes in frequency owing to loading by the titration vessel, the most important requirement is to use an oscillator circuit which is extremely stable with respect to frequency. The Clapp circuit (*6*) is a simple, one-tube oscillator with extremely high frequency stability, which is outlined schematically in Figure 4. This circuit owes its stability to relatively large capacitances in parallel with the tube capacitances. Variation of the latter due to vibration or thermal expansion have only a slight effect on the frequency.

The operation of the titrimeter is best explained by means of the block diagram of Figure 2.

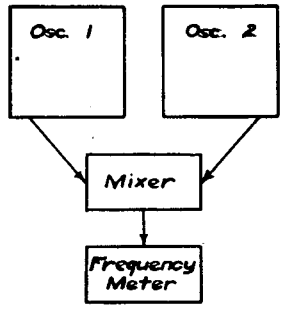

Figure 2. Block Diagram of High-Frequency Titrimeter

Oscillator 1 is a simple Clapp oscillator, with the titration beaker having two copper plates sealed to the outside placed in parallel with the tuning condenser (see Figure 4). Oscillator 2 is an identical unit, containing an identical titration beaker. The output frequencies of these two oscillators are fed into a mixer circuit, out of which is obtained a low-frequency beat (approximately 1000 to 10,000 cycles per second), whose frequency is read directly from the frequency meter.

In the 5-Mc.-per-second titrimeter, the mixer circuit was a conventional one employing a 6L7 tube (*14*). The frequency-measuring arrangement consisted of an oscilloscope and a 20- to 200,000-cycle-per-second signal generator (Hewlett-Packard, Model 200-C). The output from the signal generator was fed into the horizontal plates of the oscilloscope, and the mixer output was fed into the vertical plates. When the frequency from the signal generator was manually set equal to that from the mixer, an ellipse or circle appeared on the oscilloscope, and the mixer beat frequency could be read from the scale on the signal generator.

The description of this instrument is superficial, but this is justifiable. It is identical in principle to the 30-Mc.-per-second equipment described in the following section. A detailed account of either instrument also serves for the other. The 30-Mc.-per-second instrument is described in detail, because it is more practical and was more fully studied.

Titration Procedure. To carry out a titration, both cells were filled with identical samples of the solution to be titrated, and distilled water was added until the liquid level lay well above the copper plate electrodes. It was later found necessary to have solution in only one beaker, because frequency drift due to heating of the solution was negligible during a titration.

Mechanical stirring of the solution with a small, motor-driven borosilicate glass propeller did not interfere with stability, so long as the stirrer was kept out of the field between the copper plates.

Before starting the titration, the tuning condenser on one of the oscillators was adjusted to give a beat frequency of 1000 to 10,000 cycles per second. Operation at beat frequencies below 500 cycles per second was avoided, because there was a tendency for the two oscillators to "pull" below this frequency. Titration was then carried out by adding standard solution to one of the vessels, keeping the other unchanged throughout the titration. Standard solution was added in definite increments, and the beat frequency read after each addition.

A plot of beat frequency against volume of standard solution then gave a curve similar to that for a conductometric titration, with a break at the end point. Such a curve, with the experimental points from which it is drawn, is given in Figure 3. The end point is easily determined by the usual method of extrapolation.

Stability and Sensitivity. Inspection of Figure 3 shows only slight scattering of experimental points from the smoothed curve, and indicates the high stability of the instrument.

The sensitivity of the equipment was such that the frequency change on addition of very small increments (0.05 ml.) of standard solution could be easily measured. This change was severalfold greater than the average fluctuation or drift in beat frequency due to instrumental instability.

Differential Titration. The high sensitivity of the titrimeter, together with the high stability, allowed its use as a differential instrument as follows:

The end point was first roughly established by running rapidly through the titration, adding large increments (1 ml.) of standard solution, and observing visually when the frequency change per unit volume became suddenly different from what it had been owing to previous additions. Then, using another aliquot of the solution to be titrated, the frequency change per drop of standard solution was observed in the region of the end point.

For titrations with sharp end points, such as that in Figure 3, it was not necessary to plot the titration curve in order to locate the end point precisely, for it could be located visually within a precision of a drop or two. Many titrations do not have sharp end points: to locate end points for these, it was necessary to plot roughly the titration curve only in the region of the end point. This was the technique used for the 30 Mc. per second instrument described in the next section, and it was found much simpler and more precise than ordinary conductometric titration procedure.

Dependence of Sensitivity on Concentration and Frequency. When the titrimeter operated at about 5 Mc. per second, the concentration range for adequate sensitivity extended from 10^{-4} to 6×10^{-3} N for sodium chloride and other such salts; and for hydrochloric acid, it extended from 8×10^{-5} to 1.5×10^{-3} N. The concentration of maximum sensitivity for sodium chloride was about 2.5×10^{-3} N and for hydrochloric acid about 6×10^{-4} N.

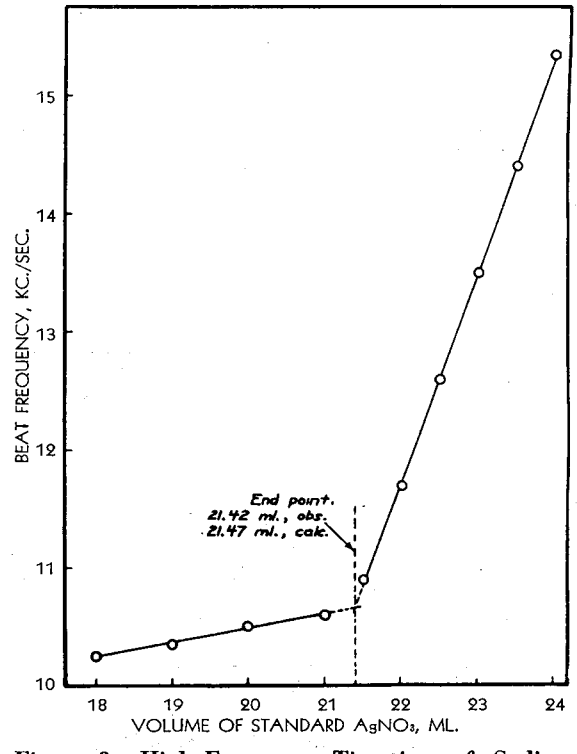

Figure 3. High-Frequency Titration of Sodium Chloride with Silver Nitrate, Using 5-Mc. Titrimeter

25 ml. of 0.01 *N* sodium chloride titrated in initial volume of 125 ml. with 0.01163 *N* silver nitrate

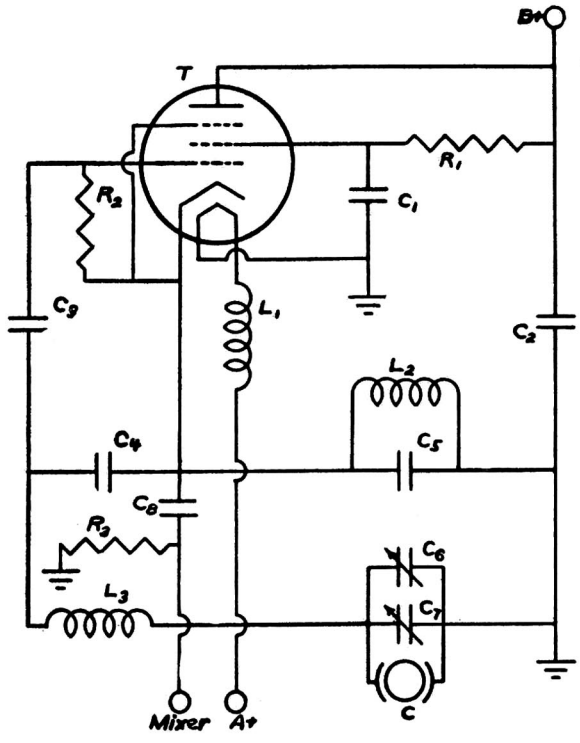

Figure 4. Schematic Diagram of 30-Mc. Clapp Oscillator

$A+$. 6 volts, high capacity, storage battery
$B+$. 300 volts, regulated
C. Condenser unit for titration vessel
C_1, C_2. 3000 $\mu\mu$f. silver-mica)
C_3. 100 $\mu\mu$f. (silver-mica)
C_4, C_5. 62 $\mu\mu$f. (ceramic, zero temperature coefficient)
C_6. Variable tuning condenser, 1.5 to 15 $\mu\mu$f.
C_7. Trimmer condenser, 1 to 4 $\mu\mu$f.
C_8. 5 $\mu\mu$f. (silver-mica)
L_1. RF choke, two layers of No. 30 wire, 60 turns, wound on plastic form with powdered iron core
L_2. RF choke, Ohmite, Z-28
L_3. 12 turns, No. 14 wire, closely wound on hollow cylinder of polystyrene, 1-inch diameter
R_1. 65,000 ohms
R_2. 15,000 ohms
R_3. 50 ohms
T. 6AC7

Using the empirical relation of Forman and Crisp (*9*), and their values for the constants for sodium chloride and hydrochloric acid (16 and 3, respectively), the concentrations at which the energy absorption of solutions of these substances is a maximum at 5 Mc. per second were calculated to be $2.7 \times 10^{-3}\ N$ and $5 \times 10^{-4}\ N$, respectively. These concentrations agree well with the concentrations of maximum sensitivity observed, and indicate the probable correctness of the mechanism of the loading effect discussed above.

Further indication of the correctness of this mechanism was obtained in two ways. First, by changing the magnitudes of the circuit elements used in the oscillators, the operating frequency was roughly doubled. The observed concentrations of maximum sensitivity were also roughly doubled, which is in agreement with the empirical relation of Forman and Crisp. Secondly, titration vessels of different volumes and different sizes of copper plates were tried. Though the sensitivity changed from one vessel to another, the concentrations of maximum sensitivity remained about the same.

Conclusions. A study of the 5-Mc. titrimeter showed that higher sensitivity and stability could be attained by measuring changes in frequency of the oscillator, rather than plate or grid currents or voltages. This instrument also gave experimental evidence of the correctness of the loading mechanism discussed earlier, and this in turn indicated the necessity of going to higher frequencies before titrations could be carried out in a higher, more practical concentration range.

In establishing these facts, the titrimeter served its purpose and was discarded. The concentration range for adequate sensitivity was too low to allow many useful titrations, and the physical arrangement required considerable manipulation during a titration. Consequently, it was not regarded as a practical analytical instrument.

THE 30-Mc. TITRIMETER

Description of the Instrument. The block diagram in Figure 2 illustrates the operation of the 30-Mc.-per-second titrimeter, briefly outlined in the previous section.

Figure 4 is a detailed schematic diagram of the Clapp circuit used in both oscillator units. Both oscillators were identically built and interchangeable. However, whereas both oscillator units contained titration cells in the 5-Mc.-per-second titrimeter, only one oscillator had a titration vessel attached in the 30-Mc.-per-second instrument. The construction of the oscillators was straightforward, except that great care was used to obtain mechanical rigidity.

Lead lengths were kept very short and the circuit components were mounted so that mechanical movement was impossible. Good mechanical construction and placement of parts were imperative for stability. The filament and plate supply leads were led through the chassis with 500-$\mu\mu$f. button-type condensers (not shown in Figure 4). The plate supply for the oscillator tubes was obtained from the same regulated B supply used for the frequency meter circuit. It was found necessary to have the amplifier for the frequency meter well isolated from the B supply. A poorly isolated amplifier required a separate B supply. A 6-volt high-capacity storage battery was used for the filaments of the 6AC7 oscillator tubes.

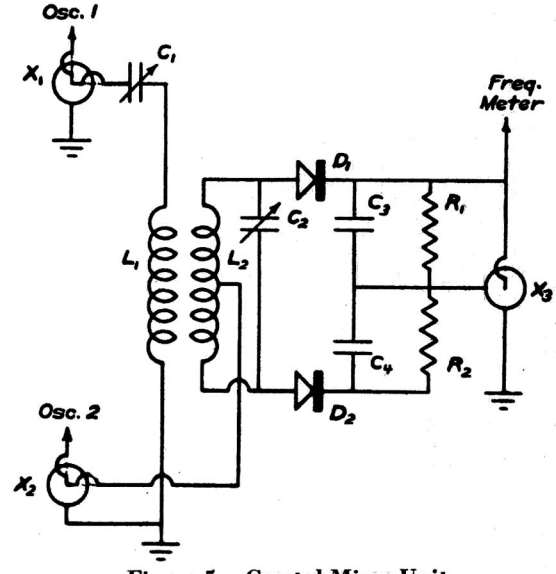

Figure 5. Crystal Mixer Unit

C_1, C_2. Variable condensers, 3 to 13 $\mu\mu$f.
C_3, C_4. 500 $\mu\mu$f.
D_1, D_2. 1N35 germanium diodes
L_1. Two sections in parallel, 35 turns each, 0.5-inch diameter polystyrene form, No. 28 wire
L_2. 17 turns, No. 28 wire, on same polystyrene form as L_1, placed between two parallel sections of L_1, 3/8 inch between coils
R_1, R_2. 35,000 ohms
X_1, X_2, X_3. Coaxial line, 50 ohms

A schematic diagram of the mixer unit is given in Figure 5. This crystal unit was found simpler and more satisfactory than the pentagrid tube unit used with the 5-Mc.-per-second titrimeter. Construction was straightforward, according to a design suggested by V. C. Rideout, professor of electrical engineering, University of Wisconsin.

This type of mixer unit isolated the two high-frequency signals and minimized the possibility of "pulling" near zero beat. The unit was completely shielded, and connections were made to the oscillators and frequency meter with coaxial cables and good, rigid, coaxial junctions. The frequency meter was converted from

a war surplus altimeter (APN 1). The basic parts were a well-isolated amplifier with automatic gain, diode limiters for converting the sine waves to square waves, a differentiator for peaking the square waves, and a milliammeter for indicating average current due to the peaked pulses after rectification (*15*). The filaments required 12-volt alternating current, and a 300-volt regulated B supply was used for the plates. The frequency input was readable directly from the milliammeter, which was adjusted to cover a range from 500 to 8500 cycles per second. This unit was more complicated than necessary.

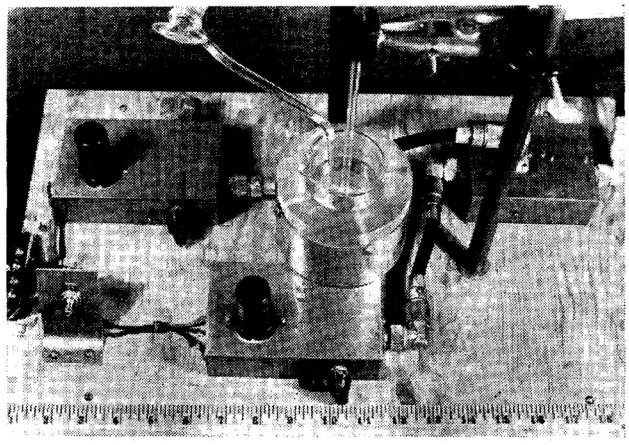

Figure 6. Thirty-Megacycle Titrimeter

There are available several commercial frequency meters working on the same principle which would serve instead of the meter described above, such as the Hewlett-Packard (Model 500-A) and the Barker and Williamson (Model 300). These would have to be preceded by one stage of amplification, inasmuch as the mixer output signal is less than 0.5 volt, which is the minimum input required for these meters.

The use of such a direct-reading frequency meter greatly reduces the manual adjustment required in the course of a titration, and is therefore much superior to the method described in connection with the 5-Mc.-per-second instrument, where an oscilloscope is used. In fact, the latter procedure is very difficult to use with the 30-Mc.-per-second instrument, because the fluctuations are of the order of 10 cycles per second.

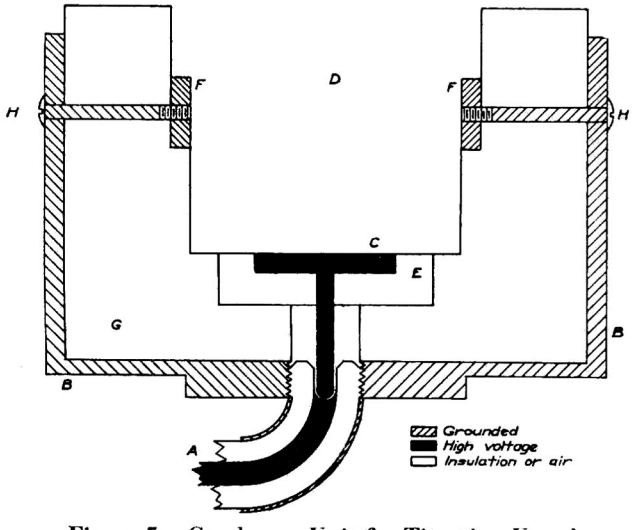

Figure 7. Condenser Unit for Titration Vessel

Vertical cross section

The oscillator and mixer units were fastened to a common plywood base on shock mounts in order to prevent relative motion with respect to each other. Before this was done, it was found that strain on the coaxial leads caused slight motion of the pins in the coaxial junctions, and this in turn caused random frequency fluctuations. The common base also held the titration stand, which contained the buret and motor stirrer.

The assembly is shown in the photograph in Figure 6. The power supply and frequency meter are not included.

The condenser unit for the titration cell was well built and rigidly mounted. Its construction is shown in the drawing in Figure 7 and the photographs in Figures 8 and 9.

A was a coaxial lead which joined the high voltage electrode, *C*, to the oscillator. This disklike electrode (1 inch in diameter) was rigidly coupled to the lead through a coaxial junction, details of which are shown in Figure 9. The electrode, *C*, was set snugly into a Teflon base, *E*, which in turn was cemented into the base of a Lucite container, *G*. This container was made from a piece of Lucite rod, bored out to accommodate a 100-ml. beaker in space *D*. The whole container and its contents were shielded with the grounded brass envelope, *B*, to eliminate effects due to body capacity. *F* was the grounded electrode of the condenser unit, consisting of a brass ring cut from brass tubing ($^1/_8$ inch in wall thickness). Details of the two electrodes are shown in Figure 8.

The inner diameter of the ring electrode was machined to allow a slip-fit for a 100-ml. beaker. Because of slight irregularities in diameter of the beaker, it could be slipped through the ring in a certain orientation, and then could be turned slightly when all the way in to allow a tight fit and to prevent motion of the beaker with respect to the ring. The ring was set on a shoulder of the Lucite container and held rigidly with three of four equally spaced set-screws, *H*. The fourth screw could be made to protrude slightly past the inner surface of the ring, and was used to accommodate beakers of slightly varying size in case of breakage. These set-screws grounded the ring to the shield.

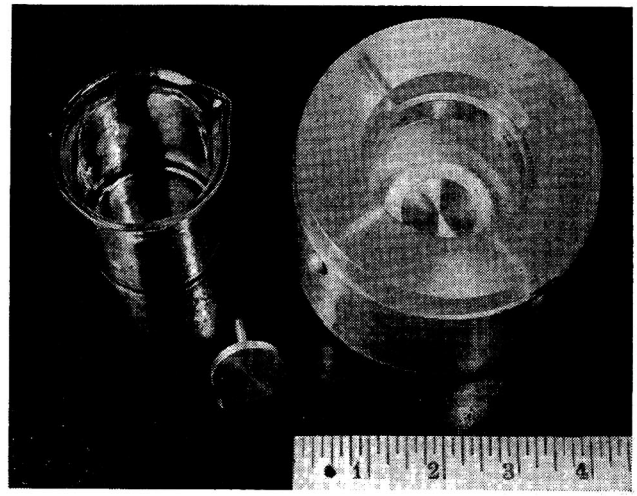

Figure 8. Condenser Unit and Titration Cell

It was necessary to prevent motion of the beaker and electrodes in order to prevent frequency fluctuations. Stability was assured by the above arrangement, and yet the beaker could easily be inserted or removed with a simple twist by gripping it around the lip, which protruded above the Lucite.

The liquid in the beaker was kept above the top edge of the ring electrode during titration. This required a minimum of 55 ml. of liquid. The stirrer, a small, motor-driven borosilicate glass propeller, was not allowed to go much below the lower edge of the ring, even though it was possible to draw a cone of air slightly below the lower edge without causing a change in beat frequency. When the stirrer was too low, or when too large a cone of air was drawn down, there was an effect on frequency which varied with the stirring speed. This was easily avoided by proper placement of the stirrer and moderate stirring speeds.

There was a small effect on frequency due to changing level as standard solution was added. This was always negligible compared to the effect due to chemical reaction, providing at least 55 ml. of liquid were present in the beaker.

Procedure for Performance of Titrations. After the beaker with solution was inserted into the high-frequency field and time allowed for warming up of the instrument, if necessary, the tuning condenser on either oscillator was adjusted for a low-frequency beat. The trimmer condenser was used to obtain the desired beat frequency more precisely. In practice, the trimmer condenser was adjusted for a beat frequency such that the frequency meter needle fell at the far end of the scale opposite to the direction in which frequency changes would occur as titration proceeded.

The meter needle would then travel over the scale as titration took place, often reaching the other end of the scale before titration was complete. The meter needle was then reset by adjusting the trimmer condenser to bring it back to the other end of the scale. A few such resettings were often necessary to complete a titration. The beat frequency was not allowed to drop below 500 cycles per per second, because there was some tendency for the oscillators to pull below this limit.

The differential titration procedure described in the section on the 5 Mc. per second instrument was used, and differential titration curves were obtained. This differential procedure virtually eliminated all error from drift due to slow temperature changes in the solution or other circuit components. It also eliminated or greatly reduced the need for laborious plotting of titration curves. Titrations with sharp end points required no plotting at all, because these end points could be established within a precision of a drop or so simply by noting the volume reading when the frequency change per drop underwent an abrupt change. Even titrations with poor end points required only rough plotting on an enlarged scale in the region of the end point (see Figures 11 to 14).

Stability of Titrimeter. The frequency stability of the Clapp oscillator (*6*) was described as 1 to 10^6. After sufficient time was allowed for warming up, it was actually found that the two oscillator units beating together gave a beat frequency with an average fluctuation of 5 cycles when operating at 30 Mc. per second. This represented a relative stability of 2 to 10^7. The time required for warming up depended on room conditions, usually taking from 15 to 45 minutes. It was easily possible to perform titrations before this time had elapsed, however, with the slight disadvantage that frequency meter readings had to be taken on top of a slow drift in frequency. This drift caused no error, as the differential method was used.

Even after the full warm-up was allowed, there was still slow drift in frequency, which was superimposed on short time fluctuations averaging about 5 cycles. This drift was shown to be due principally to temperature changes in the vacuum tubes. This drift was slow—of the order of from 0 to 20 cycles per minute—and it did not interfere at all with the differential titration procedure.

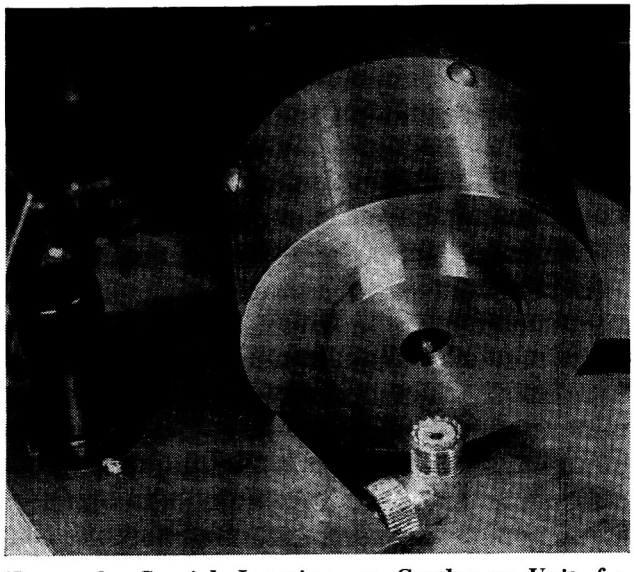

Figure 9. Coaxial Junction on Condenser Unit for Titration Cell

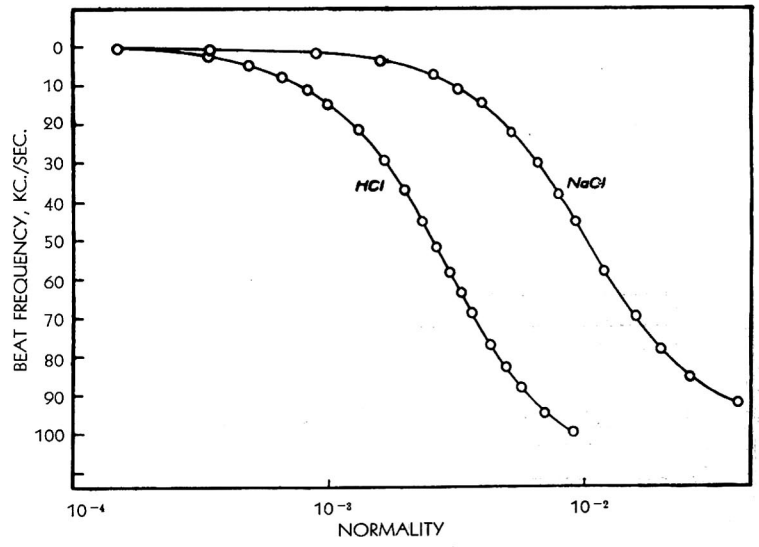

Figure 10. Effect of Concentration on Sensitivity of 30-Mc. Titrimeter

Sensitivity of Titrimeter. The fact that the 30-Mc.-per-second instrument may be used differentially shows that it has a sensitivity an order of magnitude greater than other instruments which measure plate or grid current or voltage.

A more quantitative study was made of the dependence of sensitivity on concentration than was made for the 5-Mc.-per-second titrimeter. To do this, the change in frequency was observed on adding standard hydrochloric acid to hydrochloric acid solutions of known concentration. The same was done for sodium chloride, and the results are shown in Figure 10.

In Figure 10 is plotted the beat frequency observed in the titrimeter as a function of concentration of the electrolyte in the titration vessel. The sensitivity of the instrument is appreciable only in the region where the frequency changes with concentration, and Figure 10 shows this to be so only within certain concentration ranges, which depends upon the nature of the electrolyte.

The concentrations of maximum sensitivity may be obtained from Figure 10 as approximately 0.003 *N* for hydrochloric acid and 0.014 *N* for sodium chloride. These concentrations are severalfold higher than the corresponding ones at 5 Mc. per second, and are in good agreement with those (0.003 *N* and 0.016 *N*, respectively) calculated for maximum energy absorption at 30 Mc. per second using the data of Forman and Crisp (*9*). This is further evidence of the correctness of the mechanism of loading described previously.

Titrations Performed with 30-Mc. Titrimeter. Because the 30-Mc.-per-second titrimeter possessed reasonable sensitivity at salt concentrations as high as 0.025 *N*, it was possible to investigate all the common types of reactions on which volumetric analyses are based. At least one reaction in each of the following groups was investigated: acid-base, precipitation, soluble complex formation, and redox. The titration curves are given in Figures 11 to 14, inclusive. In each case, the end point was taken as the mid-point of the steepest portion of the curve. This end point corresponded excellently with the equivalence point (based on other, accepted methods of analysis) in all titrations performed. A detailed discussion of each titration is given in the following paragraphs.

Acid-Base Titrations. In Figure 11*A*, is given the curve for the high-frequency titration of hydrochloric acid with standard barium hydroxide. The ionic reaction is

$$2H^+ + 2Cl^- + Ba^{++} + 2OH^- \longrightarrow Ba^{++} + 2Cl^- + 2H_2O$$

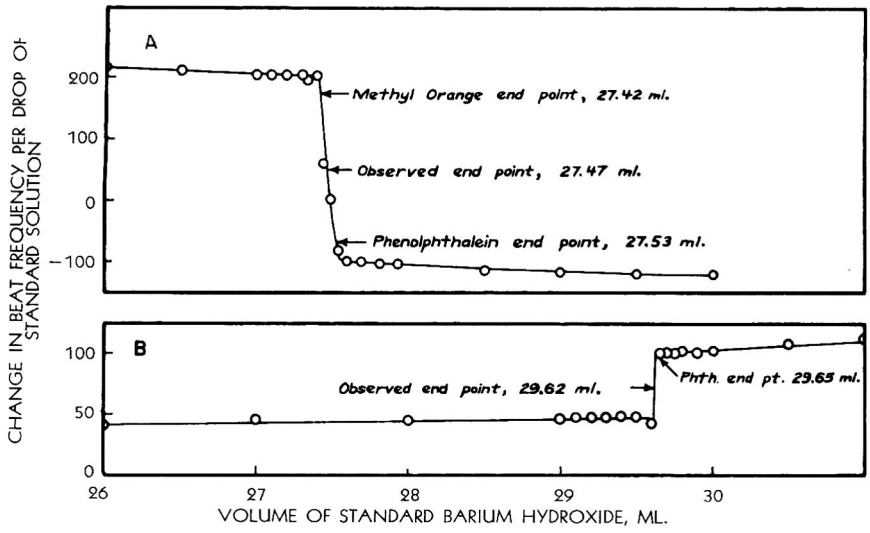

Figure 11. High-Frequency Titrations of Hydrochloric and Acetic Acids with Standard Barium Hydroxide

A. **50 ml. of 0.01 *N* hydrochloric acid titrated with 0.01820 *N* barium hydroxide**
B. **50 ml. of 0.01079 *N* acetic acid titrated with 0.01820 *N* barium hydroxide**

Per mole of standard barium hydroxide added, the effect is to replace 2 moles of hydrogen ion with 1 of barium before the equivalence point; whereas after the equivalence point, the effect is to add 1 mole of barium ion and 2 of hydroxyl. Because the dielectric constant is a function of the ion mobilities and concentrations, when a high-frequency field is applied to the solution (*8*), and the mobilities are known for many kinds of ions (*7*), the order of magnitude of the change in frequency change per drop in passing through the equivalence point is roughly predictable. For this titration, not only should the change be large, but the frequency change per drop should actually reverse in sign. This is the case, as shown in Figure 11 *A*.

In Figure 11 *B*, is given the titration curve for acetic acid with barium hydroxide.

The ionic reaction is

$$2HOAc + Ba^{++} + 2OH^- \longrightarrow Ba^{++} + 2OAc^- + 2H_2O$$

Per mole of standard barium hydroxide added, the effect is to add 1 mole of barium ion and 2 of acetate before the equivalence point; whereas after this, 1 mole of barium ion and 2 of hydroxyl are added. Because there is a good difference in mobility between acetate and hydroxyl ions, a good change in frequency change per drop is observed in passing through the equivalence point, but there is no reversal in sign.

The standard hydrochloric acid was prepared from Acculute solution (E. H. Sargent and Company). Barium hydroxide was standardized against this hydrochloric acid solution, using methyl orange and phenolphthalein as indicators. Acetic acid was standardized against the barium hydroxide using phenolphthalein. The indicator end points are annotated in Figure 11, and these agree well with the observed end points.

The indicators were not present during titrations with the high-frequency titrimeter. The color end points were determined on separate samples identical to those used in the titrimeter.

Barium hydroxide was used rather than sodium hydroxide, because it was found that the latter could not be prepared carbonate-free. Even in the titrations with barium hydroxide, carbonate could not be completely eliminated. Its presence was attested by two observations: A scum of barium carbonate was noted at the end of the titration. In Figure 11*A*, the methyl orange and phenolphthalein end points were a few drops apart.

In Figure 11*A*, the slope of the curve through the end point is not so sharp as theory demands. This, however, is not the fault of the instrument, but is due to the presence of carbonate.

Titrations Based on Precipitation Reactions. In Figure 12 are given the high-frequency titration curves for sodium chloride and potassium thiocyanate with standard silver nitrate. The ionic reactions are

$$Na^+ + Cl^- + Ag^+ + NO_3^- \longrightarrow AgCl\downarrow + Na^+ + NO_3^-$$

$$K^+ + SCN^- + Ag^+ + NO_3^- \longrightarrow AgSCN\downarrow + K^+ + NO_3^-$$

Per mole of silver nitrate added, the effect is to replace 1 mole of chloride (or thiocyanate) ion with 1 of nitrate before the equivalence point, and to add 1 mole each of silver and nitrate ions after the equivalence point. Hence, a good change in frequency change per drop of silver nitrate is observed in passing through the end point for both reactions.

Standard potassium thiocyanate and sodium chloride were prepared from Acculute solutions. Standard silver nitrate was prepared from silver nitrate as a primary standard, and doubly checked against both the thiocyanate and chloride solutions, using the Volhard (ferric ion indicator) and the Mohr (chromate indicator) methods, respectively. Excellent agreement was obtained among the standard concentrations of the three solutions. On the curves of Figure 12 are indicated the theoretical equivalence points calculated from these standard concentrations.

Agreement of observed with calculated end points is excellent. The presence of the precipitate had no effect on frequency sta-

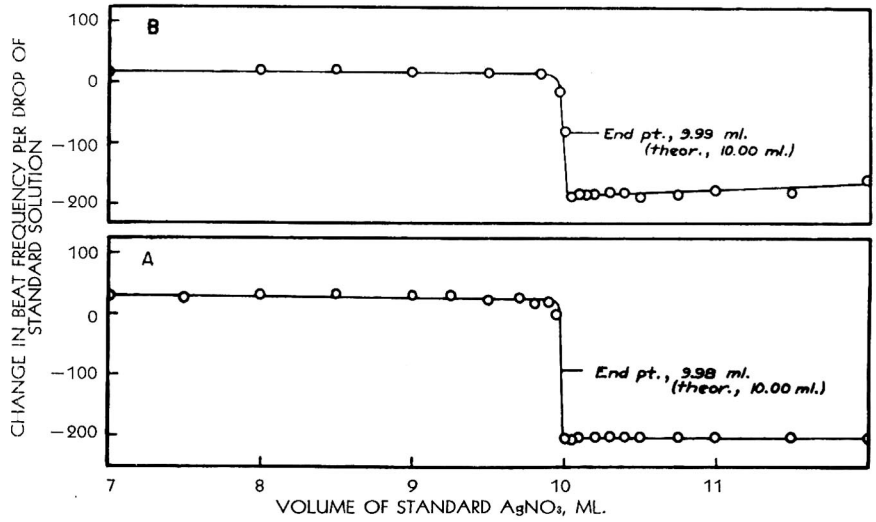

Figure 12. High-Frequency Titrations of Sodium Chloride and Potassium Thiocyanate with Standard Silver Nitrate

A. **50 ml. of 0.01 *N* sodium chloride titrated with 0.05 *N* silver nitrate**
B. **50 ml. of 0.01 *N* potassium thiocyanate titrated with 0.05 *N* silver nitrate**

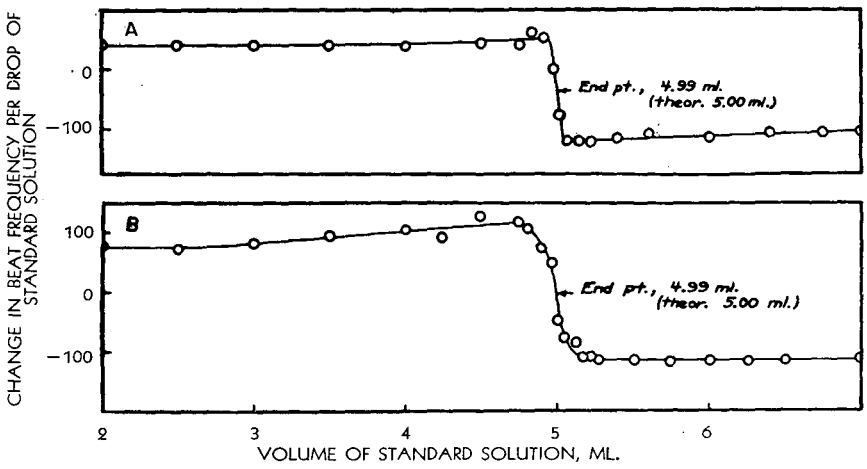

Figure 13. High-Frequency Titrations of Mercuric Nitrate with Standard Potassium Thiocyanate and Standard Sodium Chloride

25 ml. of 0.01 *N* mercuric nitrate titrated with 0.05 *N* potassium thiocyanate (*A*) and 0.05 *N* sodium chloride (*B*)

bility, and the end points were uncomplicated by adsorption effects or side reactions observed in some other types of titrations involving these substances.

Titrations Based on Formation of a Soluble Complex. In Figure 13 are given the titration curves of mercuric nitrate with standard potassium thiocyanate and standard sodium chloride. The ionic reactions are

$$Hg^{++} + 2NO_3^- + 2K^+ + 2SCN^- \longrightarrow Hg(SCN)_2 + 2K^+ + 2NO_3^-$$

$$Hg^{++} + 2NO_3^- + 2Na^+ + 2Cl^- \longrightarrow HgCl_2 + 2Na^+ + 2NO_3^-$$

Per mole of standard potassium thiocyanate (or sodium chloride), the effect is to replace 0.5 mole of mercuric ion with 1 mole of potassium (or sodium) ion before the equivalence point, and to add 1 mole each of potassium and thiocyanate (or sodium and chloride) ions after the equivalence point. Hence a good end point change is observed.

The end point for the thiocyanate reaction is very sharp because mercuric thiocyanate is highly undissociated. The end point for the chloride reaction is not so sharp because mercuric chloride is appreciably dissociated at the equivalence point. This dissociation causes considerable trouble in the selection and use of indicators for this titration (*17*). With the high-frequency method, the end point may still be located precisely.

To prepare standard mercuric nitrate solution, reagent grade red mercuric oxide as a primary standard was dissolved in an excess of nitric acid. This solution was checked against the standard potassium thiocyanate described above and good agreement was obtained. The sodium chloride used above was also used here. On the curves of Figure 13 are designated the theoretical equivalence points calculated from these standard concentrations.

Agreement of observed with calculated end points was very good. No breaks of inflections were noted at the half-way points due to formation of $HgSCN^+$ or $HgCl^+$. The usable sample size of mercuric nitrate was limited by the nitric acid present; larger samples could not be used without getting into the concentration range of decreased sensitivity.

Redox Titration. IODATE-IODIDE REACTION. In Figure 14 is given the titration of iodate with standard acid in the presence of excess potassium iodide and sodium thiosulfate, according to the method of Kolthoff (*11*). The ionic reaction is

$$IO_3^- + 6S_2O_3^{--} + 6H^+ \longrightarrow I^- + 3S_4O_6^{--} + 3H_2O$$

Per mole of standard hydrochloric acid added, the effect is to add 1 mole of chloride and to substitute 0.167 mole of iodide for 0.167 mole of iodate and 0.5 mole of tetrathionate for 1 mole of thiosulfate before the equivalence point, whereas after the equivalence point, the effect is to add 1 mole of chloride and 1 mole of hydrogen ion, most of which is probably bound with thiosulfate or tetrathionate. Hence, there may be no great change in frequency change per drop of standard acid in passing through the equivalence point. However, if there is a change it should be sharp, for this is a complete reaction.

Inspection of Figure 14 shows this to be the case: The end point change is not great, but it is sharp. Larger amounts of iodate could not be used without getting into the concentration region of decreased sensitivity.

The standard hydrochloric acid solution used was the same one used in the above titrations. The standard potassium iodate solution was prepared from potassium iodate as a primary standard and checked against the standard acid with good agreement (*11*). The theoretical end point, calculated using these standard concentrations, is indicated in Figure 14, and agrees well with the observed end point.

It was attempted to increase the magnitude of the end-point change by carrying out the reaction in the absence of thiosulfate. This reaction appeared complete enough, but the rate was too low to allow a practical titration. In fact, even in the presence of excess thiosulfate and iodide, it was noted that the reaction slowed perceptibly before the end point. In this region, it required as long as 10 to 15 seconds for each increment of standard acid to react completely. This was apparent from the behavior of the frequency meter, which changed slowly to its equilibrium value after each addition of the standard acid. This phenomenon was probably due to the low excess of thiosulfate which had to be used to remain in the region of adequate sensitivity. This reaction emphasizes the worst defect of the 30-Mc.-per-second instrument: the necessity of working in a limited, rather dilute concentration region.

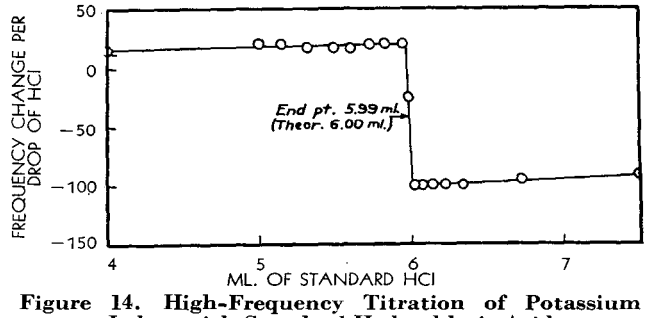

Figure 14. High-Frequency Titration of Potassium Iodate with Standard Hydrochloric Acid

2 ml. of 0.02 *M* potassium iodate titrated with 0.04 *N* hydrochloric acid in presence of 0.22 millimole of potassium iodide and 0.28 millimole of sodium thiosulfate

Conclusions Regarding 30-Mc. Titrimeter. The behavior of this instrument is well explained by the mechanism of the loading effect. This confirms the authors' belief in this mechanism.

This equipment possesses nearly all the requisites mentioned earlier for a good analytical instrument. It is very stable. It is

very sensitive in the proper concentration region. Titrations may be easily and speedily performed with it. Using the differential procedure, many titrations may be carried out with no plotting and little manipulation. Although the instrument must be carefully constructed, it is simple and easy to maintain.

The one serious difficulty with this titrimeter is the low concentration range necessary for adequate sensitivity. This property severely restricts its use in many reactions. From the mechanism of the loading effect, it is apparent that an instrument of higher frequency must be used to overcome this difficulty. The construction and properties of such instruments are the subject of the next section of this paper.

THE 360-Mc. TITRIMETER

Exploratory Work at 100 Mc. It was considered necessary to go to higher frequency in order to carry out titrations in a higher concentration range than is possible with the 30-Mc.-per-second titrimeter.

Theoretically it is possible to extend the Clapp circuit (*6*) to 100 Mc. per second. Such a unit was built, but it did not oscillate under load.

A fairly successful 100 Mc. per second unit was then constructed according to a conventional tuned plate design (*13*). The stability of this titrimeter was not sufficient for use as a satisfactory instrument. However, a few titrations were performed with it and data were obtained which showed that maximum sensitivity occurred at a salt concentration around 0.05 *N*, in accord with calculations using the empirical formula of Forman and Crisp (*9*).

It is believed that a stable 100-Mc.-per-second titrimeter could have been built, had sufficient time been spent in that direction. This appears to be close to the upper frequency limit for stable vacuum tube oscillators using conventional circuit components. Because it was desired eventually to have an oscillator operating at 300 to 500 Mc. per second, and because the 100-Mc.-per-second instrument did not seem capable of extension to this region even if it could be stabilized, work on this instrument was abandoned.

Exploratory Work on 360-Mc. Titrimeter. A survey of the electronics literature for very stable and simple oscillators operating at about 400 Mc. per second was not encouraging. A quarter-wave-length concentric line oscillator seemed the best of several kinds described (*18*), even though it was stated to possess a frequency stability of only 1 to 10^5. However, it appeared possible to improve this stability with better mechanical construction and by beating two such oscillators together, using the same filament and plate supplies, so that frequency drift would be reduced.

Two such identical oscillators were built, with the lines of such a design that they could be loaded with titration vessels. The outputs were fed into a crystal mixer, and the beat frequency was measured with the same frequency meter that was used with the 30-Mc.-per-second titrimeter. The beat frequency showed fluctuations of the order of 50 cycles per second representing a relative stability of 2 to 10^7 which was beyond expectations.

Application of this titrimeter to chemical systems is now under way. Preliminary data have shown that maximum sensitivity occurs at a salt concentration around 0.2 *M*, closely in accord with calculations using the empirical formula of Forman and Crisp (*9*). The sensitivity at present appears to be of the same order as that for the 30-Mc.-per-second instrument, with the possibility that it may be increased manyfold.

The 360-Mc.-per-second titrimeter has promise of becoming a good analytical instrument.

ACKNOWLEDGMENT

The authors wish to express their thanks to V. C. Rideout and Han Chang, of the Electrical Engineering Department, for their invaluable advice and help with this work. This work was also aided by grants from the Wisconsin Alumni Research Foundation. The authors also thank Wilbur J. Larson for providing photographs of the equipment.

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RECEIVED January 11, 1950.

Corrections

The following corrections are submitted to take care of errors in the review on "Paper Chromatography" [ANAL. CHEM., **22**, 48–59 (1950)].

The legend for Figure 2, page 50, should be "Apparatus for One- and Two-Dimensional Paper Chromatograms."

Figures 2 and 8 were accidentally interchanged, and therefore all remarks concerning Figure 2 should be referred to Figure 8, and vice versa.

Reference (*81*) should be: Gordon, Martin, and Synge, *Biochem. J. Proc.*, **37**, xiii (1943). This refers to the first reference to (*81*) on page 48. All other references numbered (*81*) should be (*39*).

DORIS L. CLEGG

In the article on "Automatic Operations in Quantitative Analysis" [Patterson, G. D., Jr., with Mellon, M. G., ANAL. CHEM., **22**, 136 (1950)], an error was made on page 143, second column, where the last paragraph should have read:

Transmissimetry. The process complementary to emission of radiant energy is transmission, and it is in this field that perhaps the largest number of applications of automatic methods exist. Tremendous advantages inherent in radiant energy transmission measurements make them especially adaptable for continuous automatic analyses. Of particular importance are the facts that the sample being tested is usually undamaged in the methods used and that the photoelectric or thermal response to changes in transmission (and thus concentration) is practically instantaneous. From the standpoint of automatization, the simplest cases, of course, are those where the desired constituent itself absorbs radiant energy of a particular wave-length band where there is negligible interfering absorption by other substances present.

Stable High-Frequency Oscillator-Type Titrimeter

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A high-frequency titrimeter using a grid-dip oscillator was developed and found to have good stability over a wide range of sensitivities. The instrument was tested using acidimetric and precipitation reactions and was found to work satisfactorily over a wide frequency range.

DURING early work sponsored by the Air Force at Oak Ridge it was found desirable to develop a sensitive volumetric instrumental procedure for the analyses of solutions containing amphoteric elements such as beryllium and aluminum. After some consideration it was decided to try the Jensen-type high-frequency titrimeter for this purpose.

High-frequency oscillators for detection of end points in volumetric chemical reactions were first used as early as 1938 by Jensen and Mosesman and Jensen and Anderson in the laboratories of the Agricultural and Mechanical College of Texas at College Station, Tex. (*1*, *5*).

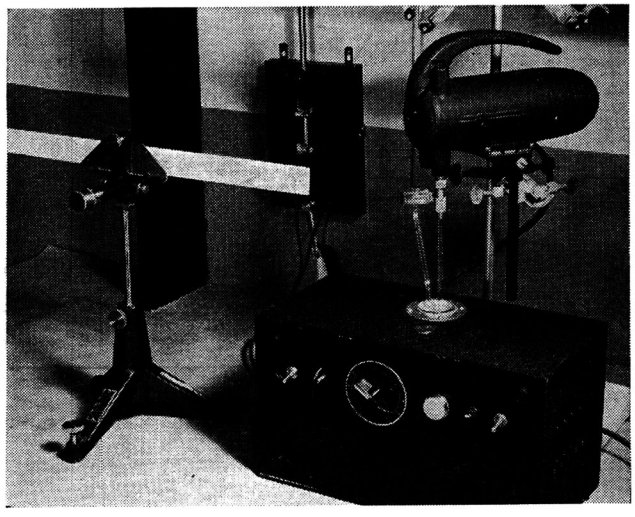

Figure 1. High-Frequency Titrimeter

Jensen and Parrock (*4*) described an instrument of the tuned grid–tuned plate type in a paper published in 1946. Blake (*3*) discussed the use of high-frequency oscillators in conductometric titrations in 1947. West, Burkhalter, and Broussard (*6*) discussed a modified design of an oscillator in a recent publication. This paper gives a rather full bibliography covering related work. Blaedel and Malmstadt (*2*) discuss a novel type of instrument.

An instrument of the type developed by Jensen and Parrock was first constructed, but was unsatisfactory because of its lack of stability when operated at high sensitivity. This feature seemed to be inherent in the instrument, inasmuch as extreme sensitivity could be achieved only by operating the tuned grid–tuned plate oscillator at a point where the first derivative of its characteristic curve was high. When the instrument was operated under these conditions it went out of oscillation too easily and hence could not be used at the extreme sensitivities needed for operations contemplated by these laboratories.

It was therefore decided to attempt development of a more stable oscillator circuit. The electronic engineers felt that a grid-dip oscillator would offer some probability of successful operation, and after some preliminary experiments such an instrument was built and tested. Figure 1 shows a photograph of the completed instrument.

[1] Present address, U. S. Atomic Energy Commission, P.O. Box 30, Ansonia Station, New York 23, N. Y.

DISCUSSION OF INSTRUMENT

The schematic diagram of the electrical circuit of the titrimeter is shown in Figure 2. This is one of many possible oscillator combinations that could be used for the titrimeter. This particular circuit was chosen because it has received considerable use in grid-dip meters and has been found to be a very dependable oscillator.

Capacities, resistances, tube types, and similar electronic data are, in general, adequately given in Figure 2. The following list of parts used in the construction of the instrument built at Oak Ridge is included as a guide in purchasing, where exact duplication of components is desired.

2 Mica condensers, 500 WVDC Centralab CC32Z
1 Thordarson 22R00 power transformer
1 resistor-RF choke combination 10 turns of No. 20 wire wound in shop around 100-ohm Allen Bradley 2-watt resistor. Wire soldered to ends of resistor
1 Thordarson T2OC59 5-henry filter choke
2 450-volt C-D No. EB9080 filter condensers
2 A-B Type J 10K potentiometers
1 wafer switch, Centralab CRL1443
1 toggle switch, Bud SW1118
1 Burgess 4FH 1.5-volt battery
Resistors as indicated on Figure 2

If it is not expedient to construct the unit with zero temperature coefficient resistors, satisfactory operation should be obtained with standard resistors if the unit is permitted to come to equilibrium temperature conditions before it is put into use. If standard resistors are used, it is recommended that 2-watt resistors be used throughout the unit. The transformer supplying power to the oscillator should have a high voltage winding of approximately 200 volts on each side of the center tap. When an oscillator circuit is wired, it is always good practice to keep grid and plate leads as short as possible. With this in mind, it is recommended that the grid and plate resistors and the grid and plate condensers be connected directly to the tube socket, and the leads to the oscillator coil and condenser plates enclosing the test tube be kept reasonable in length. The resonant circuit should be separated from the grounded chassis by a distance of at least 1 inch.

The condenser plate around the test tube consists of two brass rings mounted on a polystyrene tube. The dimensions of these rings should be determined by the size of the test tube to be used with the apparatus. A satisfactory ratio between ring diameter, length, and separation is unity—for example, if the test tube is 1.25 inches in outside diameter, the rings should be $1^5/_{16}$ inches in inside diameter and $1^5/_{16}$ inches long, and the two rings should be separated by approximately $1^5/_{16}$ inches. Any method of mounting the rings which maintains rigid spacing and concentricity and provides a high-frequency resistance path of 1 inch is satisfactory. To eliminate effects of changing volume in the sample, a grounded metallic ring should be placed above the topmost condenser ring.

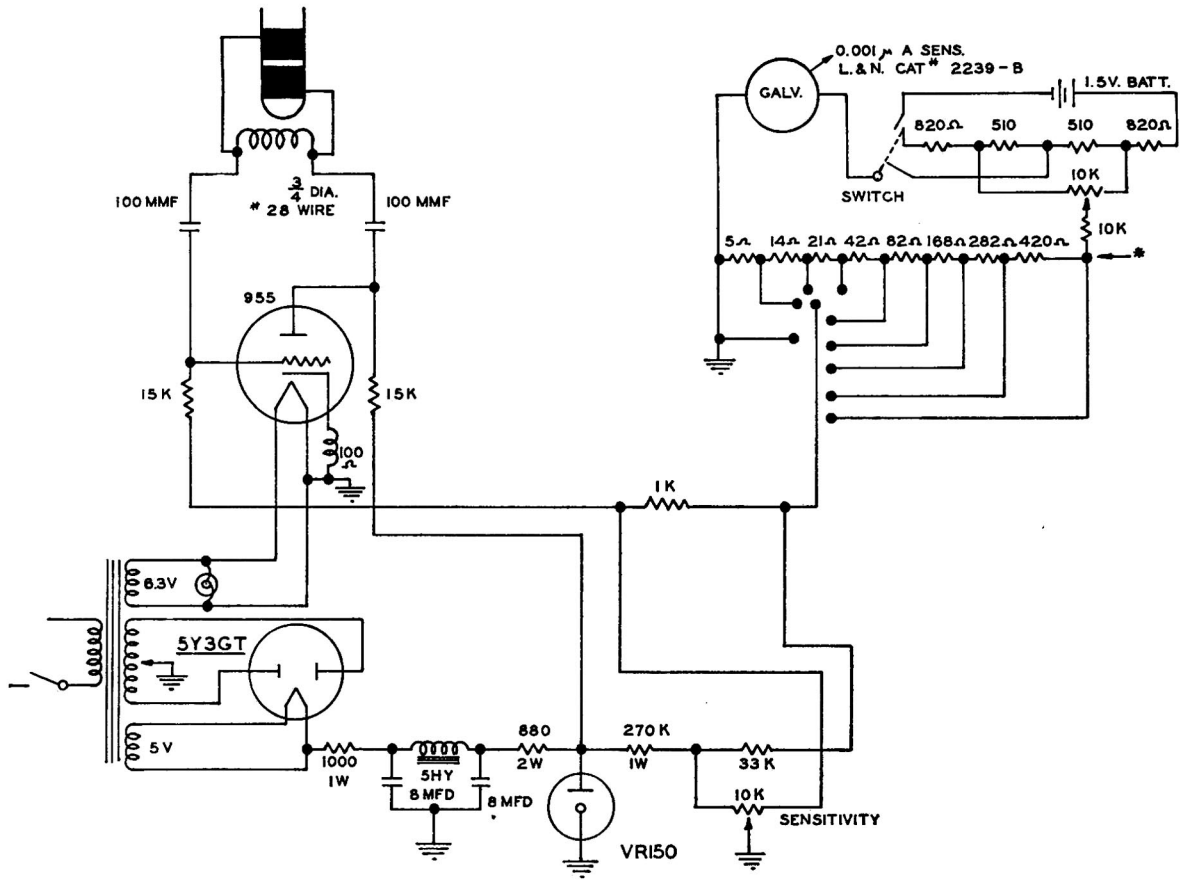

Figure 2. High-Frequency Titrimeter

Grid-dip circuit
Zero temperature coefficient resistors recommended

The tank coils used in parallel with these condensers were built in the shops and were wound on polystyrene tubes 0.75 inch in outside diameter. Plugs were soldered to the ends of the wire and rigidly fastened to the tubes in order to gain ease of replacement when changing frequencies. No. 28 varnished copper wire was used. The coils required 37 turns at 8 Mc., 21 turns at 22 Mc., and 17 turns at 35 Mc. frequency.

Operation of the oscillator is briefly described herewith. The quantity that is measured by the meter is the grid current of the oscillator. Obviously, this could be measured by a number of different kinds of meters. In order to obtain a meter with a satisfactory range of sensitivity, a standard wall-type galvanometer with the shunting resistors shown in Figure 2 was arranged in a bridge. This circuit obviously allows variations in grid current to swing the galvanometer in either direction from the previously adjusted balance point where the steady-state grid current is just balanced by the current from the 1.5-volt battery.

When an oscillator is in operation, a sinusoidal voltage $e \sin wt$ is developed across an equivalent impedance, Z, of the oscillator circuit. Figure 3 indicates the elements making up the Z of the circuit. C and R involve the liquid being studied, but L is a circuit constant. The impedance, Z, of the oscillator circuit constitutes the plate load of the vacuum tube. The gain of the vacuum tube circuit increases with an increase in the plate load. Therefore, the loop gain of the circuit will be highest when this plate load, Z, is a maximum and oscillations will be sustained for that frequency which makes Z a maximum.

$$Z = \frac{1}{\sqrt{1/R^2 + (wC - 1/Lw)^2}} \qquad (1)$$

where $w = 2\pi$ times frequency in cycles per second.

Z for any circuit is a maximum when $wC = 1/Lw$ where C is the capacity and L the inductance of the circuit. If R increases, the gain of the circuit increases and if R decreases, the gain of the

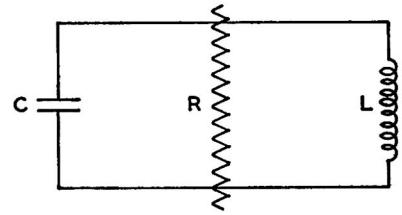

Figure 3. Fundamental Oscillator Circuit

circuit decreases. A chemical change taking place in the test tube may change C or R or both C and R of the circuit and consequently change the grid current of the oscillator.

The current in the L and C branches are 90° out of phase with the voltage—i.e., if $V = e \sin wt$—then $I_e = wce \sin (wt + \pi/2) = wce \cos wt$ and $I_2 = e/Lw \sin (wt - \pi/2) = -e/Lw \cos wt$. The

current in R is $e/R \sin wt$ and is accordingly in phase with the voltage across R. The average power dissipated by the R, L, C network is given by the expression

$$P = w/2\pi \int_0^{2\pi/w} \frac{e^2 \sin wt \sin (wt + \alpha)dt}{\sqrt{1/R^2 + (wC - 1/Lw)^2}} \qquad (2)$$

where

$$\tan \alpha = \frac{wC - 1/Lw}{1/R}$$

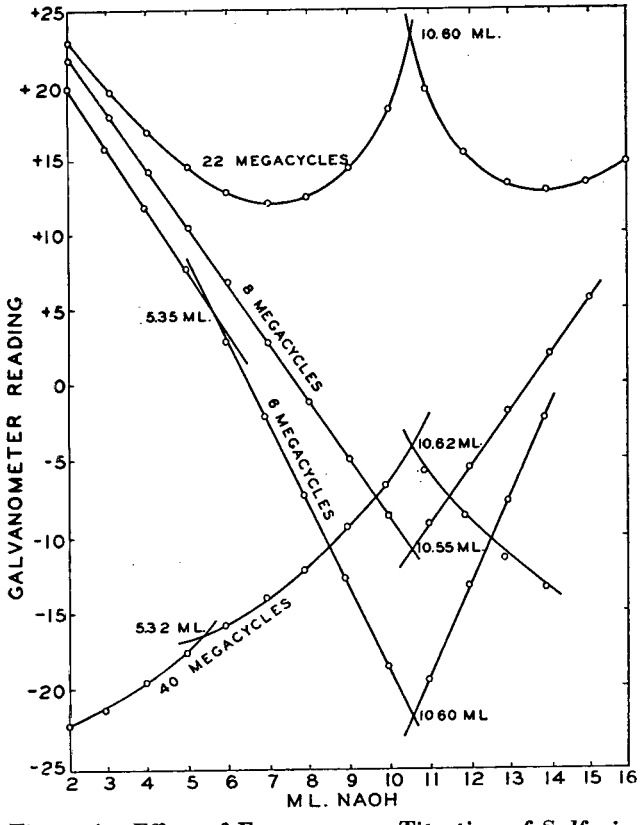

Figure 4. Effect of Frequency on Titration of Sulfuric Acid

0.1 *N* sodium hydroxide used
Theoretical end point 10.61

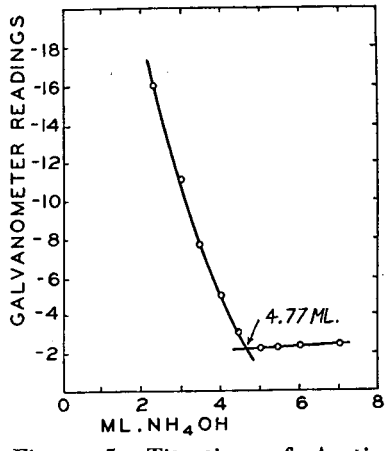

Figure 5. Titration of Acetic Acid

0.1 *N* ammonium hydroxide used
Theoretical end point 4.75

Evaluation of the power, P, by Equation 2 gives $P = e^2/2R$ (Equation 3) where e is the amplitude of the voltage across the network. Equation 3 shows that the power dissipated in the network is independent of both L and C. It depends solely upon the voltage amplitude across the network and the resistance, R, which represents the dissipative factor provided by the liquid between the condenser plates Equation 3 shows that the power dissipated in the network varies inversely with R.

The foregoing brief analysis shows that the characteristic behavior of this oscillator may change either because the capacitance of the tuned circuit changes or because the conductivity of the liquid dielectric changes. Either or both of these changes may be brought about by a chemical change in the dielectric.

OPERATION OF INSTRUMENT

After the instrument is turned on and allowed to reach thermal equilibrium, a resistance setting is chosen so that the total current change given during the complete titration is obtained over the whole range of the galvanometer. This may be found by rough preliminary runs and will hold true for all similar samples.

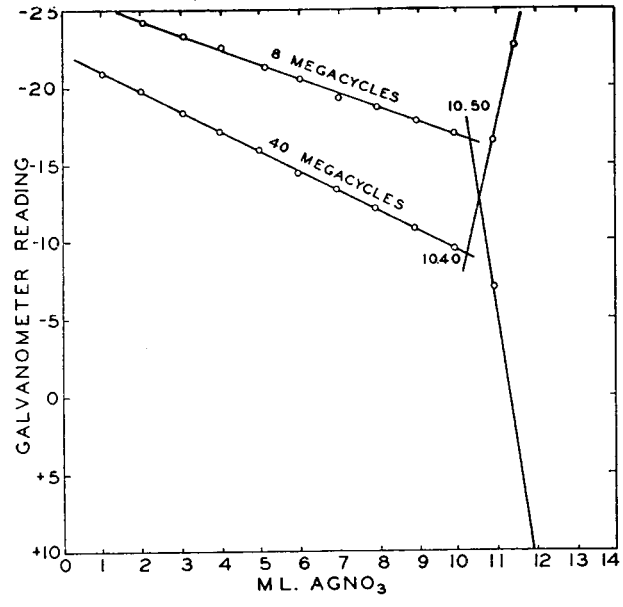

Figure 6. Titration of Sodium Chloride with Silver Nitrate

Theoretical end point 10.45

Once this is set, the current from the battery is adjusted to balance the grid current at whatever portion of the galvanometer scale the readings are to be started. Following this, the titration is performed in the normal manner and no other adjustments are required except to take readings on the galvanometer.

EXPERIMENTAL RESULTS

The titrimeter was tested using several common chemical reactions.

Acid-Base Reactions. Sulfuric acid was titrated with approximately 0.1 *N* sodium hydroxide. The titration was carried out at several frequencies, as shown in Figure 4. A midpoint break can be observed at 6 and 40 Mc. No midpoint break was observed at 8 or 22 Mc. and the latter curve shows a reversal in slope.

If proper frequencies are selected by using properly wound in-

duction coils, the instrument gives good sharply breaking titration curves for the titration of a strong acid by a strong base.

For the titration of a weak acid by a weak base, the reaction of ammonium hydroxide with acetic acid was selected. Approximately 0.1 N reagents were used. The titration curve obtained is shown in Figure 5. The titration was carried out at 8 Mc.

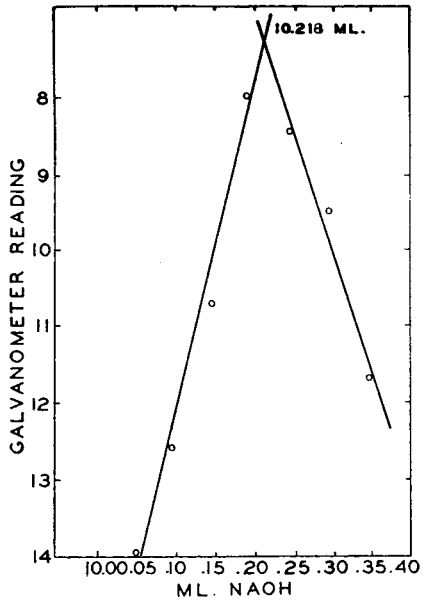

Figure 7. Obtainable Precision

Hydrochloric acid *vs.* sodium hydroxide
Theoretical end point 10.22

Precipitation Reactions. Sodium chloride was titrated with silver nitrate. Satisfactory results were achieved with this reaction at both 8 and 40 Mc. (Figure 6). A reversal of slope was obtained, but both end points are satisfactorily sharp.

Attainable Precision. In order to study the attainable precision, a titration of hydrochloric acid by 0.1 N sodium hydroxide was interrupted near the end point and carried out with a fine-tip buret with dropwise addition until the end point had been passed. The results shown in Figure 7 indicate a satisfactory precision in the determination of the end point for the majority of chemical purposes.

Numerous variables which have great significance have not been investigated or discussed in this paper. Among these is the effect of reagent concentration, limits of detection, and similar physical-chemical variables. Later work may be instituted to investigate these factors, but to date no information is available.

CONCLUSIONS

A titrimeter using a grid-dip type oscillator was constructed and found to have greater stability than other instruments tried. When tested on acidimetric and precipitation reactions, the instrument worked satisfactorily and with good precision over a wide frequency range. Additional work covering analytical reactions of interest to these laboratories is contemplated.

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Determination of Aldehydes in Presence of Ketones

Determination of Acids, Esters, and Alcohols in Presence of Aldehydes

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NUMEROUS colorimetric techniques have been proposed for the estimation of trace quantities of aldehydes. These include use of the well known Schiff's, Tollen's, and Nessler's reagents. Probably these and other selective aldehyde reagents have found their widest applicability in the determination of formaldehyde (see review by Reynolds and Irwin, *16*).

The previously reported volumetric procedures usually were based on modifications of Ripper's bisulfite method (*17*) or Romijn's alkaline iodine procedure (*19*). Although originally proposed for the determination of formaldehyde, these procedures have found limited applications in the analysis of other carbonyl compounds. The bisulfite method, reviewed in several papers (*8, 10, 15, 20*), has been used in the determination of many aldehydes and methyl ketones. An interesting variation in technique was suggested by Siggia and Maxcy (*20*) for the determination of aldehydes. These investigators employed a sulfite–sulfuric acid reagent and determined excess acid potentiometrically. Fairly sharp inflection points were observed on titration of solutions containing the aldehyde-bisulfite addition compound. With the exception of cyclohexanone, which appeared to react quantitatively, other ketones studied did not interfere seriously in the titration, provided their concentrations were below 10 mole % of that of the aldehyde.

The Romijn iodine oxidation procedure (*19*) has found restricted application in the determination of other aldehydes—for example, Korenman (*9*) and Rogers (*18*) applied this reagent to the determination of acrolein and furfural.

Another reported method of limited applicability for the determination of aldehydes was based on the Cannizzaro reaction; by employing rather drastic conditions, Palfray and co-workers (*14*) were able to determine many aromatic and a few aliphatic aldehydes.

The familiar Tollen's reagent has been employed widely in a nearly specific qualitative test for aldehydes. In this determination silver oxide, a mild oxidizing agent, is reduced by the aldehyde to free silver,

$$RCHO + Ag_2O \longrightarrow RCOOH + 2Ag \qquad (1)$$

One of the few reported quantitative applications of this reaction was described by Alekse'ev and Zvyagina (*1*) for the

The silver oxide oxidation of aldehydes has been made the basis for a quantitative acidimetric macromethod for their determination. The resulting acid is titrated with standard sodium hydroxide. With the exception of cyclohexanone, which appears to react slightly, no interference from ketones has been observed. Acids originally present in the sample consume an equivalent quantity of alkali. Some of the lower esters interfere through their saponification by the silver reagent. Methods are presented also for the determination of other compounds in the presence of aldehydes. Acids are titrated with standard sodium methylate solution. Esters are saponified after active carbonyl compounds have been converted to the oximes. Alcohols are determined after the aldehydes have been separated by treatment with silver oxide or with sodium bisulfite.

determination of 5- to 40-mg. quantities of butyraldehyde. The resulting butyric acid was oxidized to acetone with hydrogen peroxide which, in turn, was determined by an alkaline iodine (*12*), hydroxylamine hydrochloride (*2*, *11*), or furfural (*4*) procedure.

According to Equation 1, one mole of acid is formed for each mole of aldehyde group oxidized. This reaction has been made the basis of a new general acidimetric macromethod for the determination of aldehydes. The sample, containing aldehyde, is treated with an excess of silver oxide at 60° C. After oxidation is complete, the organic anions are converted to salts by the measured addition of an excess of standard aqueous sodium hydroxide. After the insoluble silver oxide has been removed by filtration, the excess sodium hydroxide is determined by titration with standard hydrochloric acid.

REAGENTS

Silver oxide, C.P., may be obtained from Eimer and Amend–Fisher Scientific Co., 635 Greenwich St., New York, N. Y.

Standard 0.5 *N* aqueous sodium hydroxide, standard 0.1 *N* aqueous hydrochloric acid, and phenolphthalein indicator solution are prepared in the usual way.

Dioxane is purified by the method of Eigenberger (*5*, *13*).

PROCEDURE FOR DETERMINATION OF ALDEHYDES

A weighed sample, containing up to 5 millimoles of aldehyde, is transferred to a 250-ml. glass-stoppered volumetric flask containing 3 grams of silver oxide and exactly 50.0 ml. of distilled water measured at room temperature (25.0 ml. of water plus 25.0 ml. of dioxane for water-immiscible samples). The firmly stoppered flask, together with a blank containing silver oxide and 50.0 ml. of solvent, is placed in a water bath at 60° C. The mixtures are shaken vigorously every 5 to 10 minutes. After an hour, the flasks are removed and allowed to cool to room temperature. Exactly 25 ml. of 0.5 *N* sodium hydroxide are added plus sufficient distilled water or dioxane to make 100.0 ml. of total liquid. The mixture is shaken and filtered. Accurately measured 25.0-ml. portions of the clear filtrate are titrated with standard 0.1 *N* hydrochloric acid to the phenolphthalein end point. There is a 1.0% contraction on mixing equal volumes of dioxane and water, with proportionately smaller contractions for intermediate mixtures. The volume of filtrate taken for titration should be corrected appropriately for the change in volume due to dioxane.

As an alternative procedure, the total liquid is recovered by filtration and the filtrate is titrated with standard 0.2 *N* hydrochloric acid. In this case careful measurement of the distilled water or dioxane is unnecessary.

The net decrease in sodium hydroxide (blank minus sample titer) is a measure of the moles of aldehyde in the sample.

Table I. Analytical Data for Aldehydes

No.	Compound	Free Carbonyl Analysis by Other Method, Wt. %	Found, Wt. %		
			Aldehyde, Ag_2O method	Free acidity	Total
1	Chloral	96.9[a]	(4)[b] 96.3 ± 0.3	...	...
2	Propionaldehyde	99.3[a]	(4) 99.5 ± 0.3	0.5	100.0
3	*n*-Butyraldehyde	98.7[a]	(4) 98.8 ± 0.3	0.8	99.6
4	*n*-Butyraldehyde[c]		(8) 100.1 ± 0.2	0.0	100.1
5	Isobutyraldehyde	96.2[a]	(3) 96.4 ± 0.2	0.9	97.3
6	α-Methyl acrolein[d]	79.5[a]	(2) 80.3 ± 0.3	0.2	80.5
7	Aldol[d]		(4) 102.1 ± 0.3	0.0	102.1
8	Crotonaldehyde	98.7[a]	(4) 98.6 ± 0.2	1.4	100.0
9	Isovaleraldehyde		(2) 96.1 ± 0.2	3.8	99.9
10	2-Ethyl hexanal[d]	89.2[e]	(8) 89.7 ± 0.4	6.6	96.3
11	Dextrose hydrate[d]		(2) 98.9 ± 0.5	0.0	98.9
12	Citronellal[d]	70.7[e]	(2) 70.9 ± 0.0	...	...
13	Benzaldehyde	89.8[a]	(12) 90.0 ± 0.5	10.1	100.1
14	Benzaldehyde[c]		(4) 99.6 ± 0.3	0.33	99.9
15	*p*-Chlorobenzaldehyde[d]		(4) 87.7 ± 0.1	11.7	99.4
16	*p*-Nitrobenzaldehyde[d]	94.4[e]	(2) 94.5 ± 0.4	2.5	97.0
17	Salicylaldehyde	98.4[e]	(4) 98.2 ± 0.3	0.5	98.7
18	Tolualdehyde		(2) 89.1 ± 0.2	10.1	99.2
19	Phenyl acetaldehyde[d]	48.7[e]	(2) 49.0 ± 0.0	...	...
20	Cinnamaldehyde[d]		(2) 61.4 ± 0.0	33.3	94.7
21	Furfural	91.2[a]	(2) 90.8 ± 0.2	1.3	92.1

[a] By hydroxylamine hydrochloride–pyridine method (*2*), 0.5 hour at room temperature.
[b] Figures in parentheses represent number of individual determinations.
[c] Freshly distilled.
[d] Practical grade. Aldehydes not marked were C.P. grade.
[e] By hydroxylamine hydrochloride–pyridine method (*2*), 2 hours at 100° C.

ANALYTICAL RESULTS

The new procedure has been used successfully for the analysis of a wide variety of aldehydes. Results on representative compounds are given in Table I. In most cases C.P. chemicals were analyzed and compared with data obtained by a hydroxylamine hydrochloride procedure. The free acid found in these samples also is recorded.

n-Butyraldehyde (No. 4, Table I) had been carefully distilled through a 36 × 0.5 inch Fenske ring-packed column which was blanketed with nitrogen. When analyzed shortly after fractionation, the heart cut of the distillate was acid-free and gave results for *n*-butyraldehyde averaging 100.1%. Benzaldehyde (No. 14, Table I) had been distilled in the same manner; however, a small quantity of acid was present in this distillate. When the per cent acid was added to the average per cent aldehyde found, the total was 99.9%.

A few aldehydes, other than those listed in Table I, were tested and found to give erratic results. A sample of 37% aqueous formaldehyde analyzed 33.6 ± 0.5% in four determinations. Acetaldehyde could not be determined by the general technique, probably because of its volatility. Values of 88 ± 1% were obtained after 1 hour at 60° C. on freshly distilled acetaldehyde, analyzing 99.8 ± 0.2% by the bromophenol blue method (*2*). Similar results were observed on samples shaken with the silver oxide for 1 hour at room temperature and then heated for 0.5 hour at 60° C. However, samples shaken continuously with reagent for 2 hours at room temperature gave recoveries of 99.0 ± 0.4%. Probably a slightly longer time at room temperature would be sufficient for complete reaction. Technical grade citral analyzed 75% compared to 92.5% by the hydroxylamine hydrochloride–pyridine method (*2*). Results on citronellal (No. 12, Table I), on the other hand, compared favorably with those of an independent method. C.P. quality anisaldehyde, vanillin, and dimethylbenzaldehyde gave values of 65, 120, and 12%, respectively. Where the total free carbonyl of those compounds listed in Table I was also determined by an independent method, the results by the new method for aldehydes checked the other results to 0.2% in nearly all cases. The data recorded in Table I indicate that the new method is applicable to aldehydes in general with an average precision and accuracy of about ±0.3%.

Several known propionaldehyde-acetone mixtures were prepared in dioxane solution. Suitable duplicate aliquots were

Table II. Determination of Acids in Presence of Aldehydes

No.	Substance	Initial Acid Found, Wt. %	Additional Acid[a], Wt. % Added	Found
1	Formaldehyde	0.28 ± 0.00	34.1	34.1
2	Butyraldehyde	3.53 ± 0.01	28.6	28.5
3	Butyraldehyde[b]	0.00 ± 0.00	16.2	16.2
4	Isovaleraldehyde	9.62 ± 0.01	35.9	35.8
5	Benzaldehyde	10.83 ± 0.02	38.3	38.3
6	Benzaldehyde[c]	0.33 ± 0.00	16.4	16.5
7	p-Tolualdehyde	13.96 ± 0.02	29.7	29.6
8	Dimethylbenzaldehyde	0.74 ± 0.00	35.8	35.9

[a] Added acid was acetic.
[b] Freshly distilled in an atmosphere of nitrogen.
[c] Freshly distilled.

analyzed by the standard procedure. The following are typical results:

Acetone, Wt. %	Propionaldehyde, Wt. % Added	Found
96.5	3.5	3.6 ± 0.2
88.6	11.4	11.2 ± 0.2
55.0	45.0	45.0 ± 0.1
33.3	66.7	67.0 ± 0.3
11.5	88.5	88.3 ± 0.2

DETERMINATION OF ACIDS, ESTERS, AND ALCOHOLS IN PRESENCE OF ALDEHYDES

Interfering Substances. The new method is subject to interference from peroxides, sodium methylate, acids, and esters. No interferences were observed from alcohols, ethers, and most ketones. Acetone, methyl ethyl ketone, diethyl ketone, and acetophenone did not react. However, cyclohexanone appeared to react slightly. Quadruplicate analyses by the aldehyde method of peroxide-free cyclohexanone gave values of 1.5 ± 0.2%. Peroxides in general probably interfere—for example, cyclohexanone containing 1% peroxide (calculated as H_2O_2) gave apparent aldehyde values as high as 25%. Sodium methylate in methanol solution reacted with the silver oxide reagent; a lustrous silver mirror was plated on the glass container. The observed behavior suggests the following reaction:

$$NaOCH_3 + 2Ag_2O \longrightarrow 4Ag + HCOONa + H_2O \qquad (2)$$

Acids interfere directly in the aldehyde procedure by consuming an equivalent quantity of alkali. Therefore, the sodium hydroxide titer must be corrected for the quantitative acid reaction. Acids cannot be determined accurately in the presence of active aldehydes by titration with aqueous sodium hydroxide. Several aldehydes were titrated with aqueous sodium hydroxide. No stable phenolphthalein end points could be obtained and in general the results were 0.3 to 0.6% high. However, when acid-aldehyde mixtures are titrated with standard sodium methylate in dry methanol, sharp phenolphthalein end points are obtained which are a quantitative measure of the total acidity of the sample.

Duplicate 4- to 10-gram portions of the aldehydes listed in Table II were titrated with 0.5 *N* sodium methylate to determine their initial acid contents. Then larger known amounts of acid were added to separate portions of the aldehydes and the solutions titrated using the acid titer of the initial aldehyde as the blank.

Determination of Acids in Presence of Aldehydes. REAGENTS. Approximately 0.5 *N* sodium methylate is prepared by dissolving 27 grams of the C.P. powder (Mathieson Alkali Works) in 1 liter of dry methanol solution. The reagent is standardized against Bureau of Standards benzoic acid or standard 0.5 *N* aqueous hydrochloric acid.

Phenolphthalein indicator solution.

PROCEDURE. The sample, containing up to 20 millimoles of acid, is transferred to an Erlenmeyer flask and titrated with standard 0.5 *N* sodium methylate to the phenolphthalein end point.

The more easily hydrolyzed esters are saponified by the silver oxide reagent of the procedure for aldehydes. For example, ethyl acetate, methyl propionate, methyl valerate, and cyclohexyl acetate reacted quantitatively in either aqueous or water-dioxane solution (see also Table IV). Therefore, the net alkali consumed in the aldehyde determination must be corrected for the ester equivalent. This ester equivalent can be determined accurately only for esters which are quantitatively saponified by the silver oxide reagent.

Direct saponification methods cannot be used for the determination of esters in the presence of aldehydes. Aldehydes seriously interfere by absorbing alkali in amounts which usually bear no stoichiometric relation to the concentrations of aldehydes. The behavior of aldehydes toward heating with sodium hydroxide is illustrated by the following duplicate analyses of several aldehydes, which contained less than 0.5% of ester. The Bryant-Smith saponification technique (*3*) was used which involves heating the sample at 60° C. for 30 minutes with 2 *N* sodium hydroxide in 90% methanol.

Aldehyde (≥ 99.5 Wt. %)	Apparent Ester (as Wt. % C_8 Ester)
Propionaldehyde	21.0 ± 0.2
Isobutyraldehyde	23.6 ± 0.3
Benzaldehyde	18.0 ± 0.5
Dimethylbenzaldehyde	1.0 ± 0.1

However, Smith and Bryant (*22*) found that the oximes of aldehydes were sufficiently stable toward alkali to permit esters to be saponified without interference. Although ketones did not interfere in the saponification, it proved easier not to attempt to distinguish between aldehydes and the more active ketones where both were present, but to convert both to the corresponding oximes, using aqueous hydroxylamine as the reagent. It was necessary to avoid excess hydroxylamine, because the free base in the presence of strong alkalies reacts with esters to form acyl hydroxamic acids,

$$H_2NOH + RCOOR' \longrightarrow RC(OH)=NOH + R'OH \qquad (3)$$

The hydroxamic acids are too weak to be titrated successfully. When permitted to form, they introduce a considerable error into the ester determination. The correct amount of hydroxylamine to be used is predetermined by the hydroxylamine-pyridine method (*2*).

Determination of Esters in Presence of Aldehydes. REAGENTS. Hydroxylamine hydrochloride reagent, 2 *N*, is prepared from 138 grams of C.P. salt in sufficient distilled water to make 1 liter of solution. The exact normality of the 2 *N* hydroxylamine hydrochloride is determined by titrating the free acidity in a 20.0-ml. portion of the solution to the bromophenol blue end point with aqueous 0.5 *N* sodium hydroxide, then adding an excess of C.P. acetone (6 to 10 ml.), and allowing the mixture to stand 5 minutes. Finally the solution is again titrated to the bromophenol blue end point with standard aqueous 0.5 *N* sodium hydroxide. The latter alkali titer determines the normality of the reagent solution, one mole of hydroxylamine hydrochloride being equivalent to a mole of sodium hydroxide.

As an alternative method for determining the hydroxylamine reagent normality, a second 20-ml. portion may be titrated to the phenolphthalein end point. The net titer between the bromophenol blue and phenolphthalein end points also is a direct measure of hydroxylamine hydrochloride.

Thymolphthalein indicator is a 1% solution of the C.P. material in absolute ethanol.

Approximately 2 *N* sodium hydroxide reagent is prepared by dissolving 80 grams of C.P. pellets in sufficient 90% methanol–10% water to make 1 liter of solution.

PROCEDURE. A carefully measured volume of sample is weighed into a 250.0-ml. volumetric flask or Pyrex brand pressure bottle. The amount of active carbonyl is determined by means of the hydroxylamine-pyridine method (*2*), allowing a 5-minute reaction time at room temperature.

Then the calculated equivalent amount of 2 *N* aqueous hydroxylamine hydrochloride solution is delivered accurately into an empty 250.0-ml. glass-stoppered volumetric flask by means of a 10.0-ml. microburet. Thymolphthalein is added and the solution is carefully neutralized to a blue color with 2 *N* sodium hydroxide in 90% methanol (10% water). The solution is next

back-titrated with a few drops of 0.5 *N* aqueous hydrochloric acid until the blue color just disappears. The hydroxylamine is then substantially all in the free form. The sample to be analyzed is now added. For optimum precision and accuracy, this sample should contain about 10 milliequivalents of acid plus ester. The sample also contains aldehyde plus ketone exactly equivalent to the hydroxylamine liberated in the above step. The mixture is allowed to stand for 5 minutes at room temperature. Then 20.0 ml. of 2 *N* sodium hydroxide are added, and the flask is stoppered and heated for 0.5 hour in a water bath at 60° C. At the end of this time, the flask is removed, cooled, and titrated with standard 0.5 *N* hydrochloric acid. [These last steps are essentially identical with the previously published general saponification procedure (*3*).] The thymolphthalein color change is sharper in the presence of oximes than the phenolphthalein end point.

At least one blank, containing 20.0 ml. of the 2 *N* alkali, is run with each set of samples. No hydroxylamine should be employed in the blank, because the free base undergoes decomposition when heated in strong sodium hydroxide solutions.

The reagents employed prior to the saponification step are used in concentrated form to avoid excessive dilution. In general, the final caustic normality for saponification should be no less than 1 *N*.

A number of synthetic mixtures were analyzed successfully by the above procedure. Typical results are given in Tables III and IV.

Most of the samples of Table III were prepared volumetrically and then calculated to a weight basis by means of densities. Nos. 3, 4, 10, and 11 were prepared on a gravimetric basis. The data of Table III have an average reproducibility of about ±0.3% in weight composition and seldom differ from the true composition by more than 1%. The largest errors are associated with the volumetric samples containing relatively small concentrations of ester and probably represent, in part, errors in volume measurement.

A series of known mixtures of isovaleraldehyde and methyl valerate was prepared and analyzed by both the silver oxide method (aldehyde plus ester) and the modified saponification procedure. Two 100-ml. dioxane solutions were prepared. One contained 8.92 grams of isovaleraldehyde and the other 14.0 grams of methyl valerate. Aliquants of each were mixed and analyzed. Results are given in Table IV.

The ester-free aldehyde was analyzed by the 2.5 pH hydroxylamine hydrochloride method (*23*). In general, the accuracies of the found values for both aldehyde and ester compare favorably with those data of Table I and III.

Aldehydes interfere in most acylation procedures for alcohols. [A notable exception is the phthalic anhydride method of Elving and Warshowsky (*6*), which is applicable to many primary and secondary alcohols.] The silver oxide method frequently may be employed conveniently for the removal of aldehydes from samples prior to the determination of free alcohols, except in the presence of easily hydrolyzed esters.

Determination of Alcohols in Presence of Aldehydes. The sample, containing up to 10 milliequivalents of aldehyde but no more than 20 milliequivalents of C_1–C_4 alcohol, is weighed into a distillation flask containing about a 100% excess of silver oxide (4.6 grams of Ag_2O for 10 millimoles of aldehyde). Fifty milliliters of water are added and the mixture is heated for 0.5 hour at 60° C. At the end of this time, the sample flask is removed and connected to a small still. If the alcohol is methanol or ethanol, 50 ml. of C.P. benzene are added and the mixture is distilled carefully until 5 to 10.0 ml. of benzene-water azeotrope have been removed.

Azeotropic Data (*7*)

Benzene-methanol	B.P. 58.34° C., 39.6% CH_3OH
Benzene-ethanol	B.P. 68.24° C., 32.4% C_2H_5OH
Benzene-water	B.P. 69.25° C., 8.8% H_2O

The distillate is diluted to exactly 50.0 ml. with purified dioxane and suitable aliquots are used for the subsequent hydroxyl determination. If the alcohol is propanol or butanol, the aqueous silver oxide–treated sample mixture is distilled directly until 25.0 ml. of distillate have been obtained. Then 25.0 ml. of C.P. benzene are added to the distillate plus sufficient anhydrous potassium carbonate to saturate the water layer. The benzene layer, which now contains essentially all of the alcohol, is removed and used in the analysis.

Alcohols containing five carbon atoms or more are only slightly soluble in water and are best separated by benzene extraction of a bisulfite-treated aqueous solution. The sample is shaken vigorously with sufficient 10% aqueous sodium bisulfite solution to react with all of the aldehyde. Usually a 50% molar excess of bisulfite solution is adequate. Then the mixture is extracted with 25-ml. portions of benzene. The hydrocarbon extracts, which now contain all of the alcohol, are combined and measured portions are used for the alcohol analysis.

Typical results on several aldehyde-alcohol mixtures are given in Table V. In all cases the acetyl pyridinium chloride method (*21*) was used for the determination of the alcohol.

DISCUSSION

The new procedures have been used successfully for the analysis of complex mixtures involving aldehydes and for the removal of aldehydes to prevent their interference in analyses for other functional groups.

Table III. Determination of Esters in Presence of Aldehydes

No.	Mixture as Prepared: Aldehyde, wt. %		Ester, wt. %	Ester by Analysis, Wt. %
1	Propionaldehyde	95.0	5.0[a]	(3)[b] 6.7 ± 0.2
2	Propionaldehyde	43.5	56.5[c]	(3) 56.8 ± 0.3
3	*n*-Butyraldehyde	95.0	5.0[d]	(2) 5.4 ± 0.2
4	*n*-Butyraldehyde	54.5	45.5[d]	(3) 45.2 ± 0.3
5	Isobutyraldehyde	94.9	5.1[a]	(6) 6.2 ± 0.5
6	Isobutyraldehyde	78.8	21.2[a]	(2) 22.0 ± 0.3
7	Isobutyraldehyde	43.1	56.9[c]	(3) 57.6 ± 0.3
8	Citral	87.4	12.6[a]	(3) 13.7 ± 0.4
9	Benzaldehyde	96.0	4.0[a]	(3) 3.3 ± 0.2
10	Benzaldehyde	94.0	6.0[e]	(4) 6.2 ± 0.3
11	Benzaldehyde	65.0	35.0[e]	(3) 34.8 ± 0.3
12	Benzaldehyde	38.0	62.0[a]	(3) 61.3 ± 0.3
13	Dimethylbenzaldehyde	95.9	4.1[a]	(3) 3.7 ± 0.4
14	Dimethylbenzaldehyde	37.2	62.8[b]	(3) 61.9 ± 0.2

[a] Isobutyl isobutyrate.
[b] Figures in parentheses represent number of individual determinations.
[c] Di-*n*-butyl phthalate.
[d] Ethyl acetate.
[e] Phenyl acetate.

Table IV. Analysis of Isovaleraldehyde–Methyl Valerate Mixtures

Added, Weight %: Aldehyde	Ester	Acid	Found, Weight %: Aldehyde[a]	Ester
95.7	0.0	4.3[b]	95.8	0.3
89.0	7.0	4.0	88.8	5.9
87.9	8.2	3.9	88.2	8.6
54.7	44.1	1.2	54.5	45.0
53.5	45.4	1.1	53.5	46.3
38.3	61.1	0.6	37.8	61.0
13.9	85.8	0.3	13.5	85.5
9.1	90.6	0.3	8.7	90.8
4.6	95.2	0.2	4.2	95.1
0.0	99.9	0.1[b]	−0.2	99.9

[a] Corrected for known ester added.
[b] Determined by titration of concentrated sample; all other acid values were calculated.

Table V. Determination of Alcohols in Presence of Aldehydes

Aldehyde, Weight %		Method for Aldehyde Removal	Alcohol, Weight %:	Added	Found
Propionaldehyde	30.0	Ag_2O	Methanol	70.0	68.9
	73.5			26.5	26.0
Propionaldehyde	64.3	Ag_2O	Ethanol	35.7	35.0
Butyraldehyde	47.5	Ag_2O	Propanol	52.5	51.7
Butyraldehyde	24.7	Ag_2O	Butanol	75.3	74.8
	51.2			48.8	48.4
	78.3			21.7	21.3
Butyraldehyde	15.0	$NaHSO_3$	Pentanol	85.0	84.6
	73.6			26.4	26.1
Valeraldehyde	45.7	$NaHSO_3$	2-Ethylhexanol	54.3	54.0
3,5,5-Trimethylhexanal	9.6	$NaHSO_3$	3,5,5-Trimethylhexanol	90.4	90.3
	28.5			71.5	71.3
	58.7			41.3	41.2
	97.3			2.7	2.4

A synthetic mixture containing 50% methyl acetate, 12% methyl formate, 28% acetone, and 10% propionaldehyde analyzed 9.8 ± 0.4% aldehyde after correction for the ester interference. Another mixture containing 60.0% acetic acid, 12.0% methyl formate, 24.0% methyl acetate, 3.0% acetone, and 1.0% propionaldehyde was first neutralized with sodium methylate and then analyzed. Values of 1.0 ± 0.5% propionaldehyde were found, after correction for the saponification of the esters.

The silver oxide procedure also was employed to permit the determination of acetone in the above complex mixtures. Portions of both samples were treated with silver oxide according to the standard method. Then the acetone was separated by distillation through a simple column packed with Pyrex brand glass rings, sufficient water being taken over to assure complete removal of acetone. Analyses of the distillate by the 2.5 pH hydroxylamine hydrochloride method (*23*) gave values of 27.3 ± 0.3% (added 28.0%) and 3.0 ± 0.2% (added 3.0%) acetone.

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Determination of Carbonyl Compounds in the Presence of Organic Acids

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A modified acidimetric oximation procedure is presented, in which the pH of the reagent is reduced to a point where carboxylic acids barely interfere—e.g., only 0.05 ml. of 0.5 *N* sodium hydroxide is consumed per gram of most carboxylic acids. The hydroxylamine hydrochloride reagent is prepared in 80% ethanol solution, thymol blue indicator is added, and the apparent pH is adjusted to 2.50 by the addition of aqueous hydrochloric acid. Most aldehydes and methyl and ethyl ketones react quantitatively at room temperature. The ketoximes are sufficiently basic at pH 2.50 to interfere slightly and, therefore, to require a small correction factor. This system also may be employed to determine certain acetals and ketals. In many cases the hydrogen ion concentration of the reagent is sufficiently high to effect nearly quantitative hydrolysis of these combined carbonyl compounds in 1 to 2 hours at 98° to 100° C. Thus a scheme is available for determining in a single sample both free and combined carbonyl compounds. After titration for the free carbonyl groups, the sample plus reagent is heated on the steam bath, cooled, and titrated again. The second titer is a direct measure of acetals or ketals.

HYDROXYLAMINE hydrochloride is a reliable and widely applicable reagent for the determination of aldehydes and ketones. In the equations

$$RCHO + H_2NOH.HCl \longrightarrow RC(H)=NOH + H_2O + HCl \quad (1)$$

and

$$RCOR' + H_2NOH.HCl \longrightarrow RR'C=NOH + H_2O + HCl \quad (2)$$

one mole each of acid and water is released for each equivalent of carbonyl compound reacted. Originally this reagent was used by Brochet and Cambier (*1*) for the quantitative determination of formaldehyde. Later investigators demonstrated that hydroxylamine hydrochloride (or sulfate) can be the basis of valuable procedures for the determination of all types of carbonyl compounds. These procedures, summarized by Mitchell and Smith (*7*), almost invariably involved titration of the acid freed during oxime formation according to Reactions 1 and 2. In one case the water of reaction was determined by titration with Karl Fischer reagent (*8*). For trace quantities of acetone, differential pH measurements have been employed (*3*, *5*).

All the acidimetric methods previously reported were subject to interference from acidic and basic components of the samples to be analyzed. However Eitel's (*4*) potentiometric titration method reportedly could tolerate small quantities of acids. For a nearly completely aqueous system, this investigator assigned a pH of 4.1 as the equivalence point for free hydrochloric acid in the presence of hydroxylamine hydrochloride. In carrying out an analysis Eitel (*4*) added aqueous hydroxylamine hydrochloride (or sulfate) to an alcoholic solution of the sample. Depending on the type of carbonyl compound, the mixture was

allowed to stand at room temperature or heated on a steam bath. Then, after dilution with water, the precipitated oxime was removed by filtration and the resulting solution was titrated with standard sodium hydroxide to a pH of 4.1. If acids were present, Eitel (*4*) neutralized the alcoholic solution of the sample to phenolphthalein before addition of the hydroxylamine reagent.

With the exception of acetone, all the reported analyses were of aldehydes. Eitel's ability to titrate free acids with aqueous sodium hydroxide in the presence of aldehydes is surprising. Sodium salts of carboxylic acids should interfere in the subsequent determination of carbonyl compounds. Hydrochloric acid freed during oxime formation would tend to displace the acyl group of the sodium salt (Equation 3), but the resulting organic acid would not be titrated stoichiometrically at pH 4.1.

$$RCOONa + HCl \longrightarrow RCOOH + NaCl \qquad (3)$$

A new procedure has been developed to fill the need for a rapid and simple method for the direct determination of carbonyl compounds in the presence of high concentrations of carboxylic acids. To accomplish this objective, a reagent of hydroxylamine hydrochloride in 80% ethanol is used. The acidity of the hydroxylamine hydrochloride solution is adjusted to an apparent pH of 2.50 where carboxylic acids scarcely interfere but where the titration of hydrochloric acid, freed from its hydroxylamine salt, is essentially stoichiometric. The strongly alcoholic environment serves as a solvent for the carbonyl compound and its oxime. This permits the use of larger samples than are practical in an aqueous system. The titration can be made either visually or potentiometrically. The environment appears to improve the sharpness of the end point over that observed in an aqueous system containing no organic solvents at the low pH used. The new method combines speed with high precision and accuracy for the analysis at room temperature of most aldehydes and many methyl and ethyl ketones.

Acetals, the condensation products of one equivalent of aldehyde with two equivalents of alcohol, are subject to acid hydrolysis.

$$RCH(OR')_2 + H_2O \xrightarrow{H^+} RCHO + 2R'OH \qquad (4)$$

For the determination of some acetals, Siggia (*13*) recommended acid hydrolysis at room temperature followed by reaction with sodium sulfite. Siggia also discussed the application of an aqueous hydroxylamine hydrochloride reagent adjusted to a pH of 3.1 to the analysis of many acetals, ketals, and vinyl ethers. In this method, limited quantities of alcohol were added only if necessary for solution of the sample. This method requires the use of a pH meter. At a pH of 3.1 organic acids would titrate partially.

The new 2.5 pH hydroxylamine hydrochloride reagent is sufficiently acid to effect at 100° C. essentially complete hydrolysis of many linear acetals. At room temperature usually only free carbonyl groups react with the reagent. The hydrolysis at 100° C. probably is aided by the presence of hydroxylamine hydrochloride which acts as a carbonyl acceptor. The 2.5 pH reagent thus provides a convenient means for the analysis of many acetals even in the presence of free carbonyl groups.

REAGENTS

One liter of 0.5 *N* hydroxylamine hydrochloride in 80% ethanol is prepared by dissolving 35 grams of Eastman Kodak No. 340 hydroxylamine hydrochloride in 160 ml. of distilled water and diluting to volume with 95% ethanol. Then 6 ml. of 0.1% thymol blue solution in dry methanol are added plus sufficient (usually about 6 ml.) 0.5 *N* aqueous hydrochloric acid to adjust the apparent pH to 2.50 ± 0.01.

In this laboratory the hydroxylamine reagent is prepared in 18-liter quantities. Before pH adjustment, 15 ml. of 6 *N* aqueous hydrochloric acid are added. Then a 100-ml. portion of reagent is transferred to a beaker. A glass-calomel electrode pair is placed in the solution and 0.5 *N* hydrochloric acid is added carefully until the meter registers a pH of 2.50. The calculated quantity of 0.5 *N* hydrochloric acid is added to the remaining reagent. After thorough mixing the pH of the final reagent is checked on another 100-ml. portion and, if necessary, the acidity is readjusted. The reagent may be stored indefinitely, although occasional checks on the pH should be made.

Thymol blue indicator solution is made by grinding 1 gram of the powder (Lamotte) with 4 ml. of 0.5 *N* sodium methylate in methanol. The resulting paste is diluted with methanol to 1 liter.

Approximately 0.5 *N* sodium hydroxide is prepared by dissolving 80 grams of C.P. pellets in sufficient 90% methanol (10% water) to make 4 liters of solution. The solution is stored in a reservoir connected to a 50- to 100-ml. buret graduated in 0.1 ml. Because methanolic solutions exhibit significant coefficients of expansion, the solution temperature should be noted at the time of titration and the appropriate temperature correction applied to the normality. The reagent should be standardized at regular intervals against standard benzoic acid or standardized 0.5 *N* aqueous hydrochloric acid.

One-liter quantities of 90% methanol are prepared by mixing 900 ml. of nearly dry methanol with 100 ml. of distilled water.

PROCEDURES

Determination of Free Carbonyl Groups. VISUAL TITRATION. Using a suitable dispensing pipet, 100 ± 1 ml. of hydroxylamine reagent are delivered into a 250-ml. volumetric flask or 350-ml. (12-ounce) Pyrex brand pressure bottle (Code No. 424361, Corning Glass Works). Then the sample, containing up to 25 milliequivalents of aldehyde plus ketone, is weighed into the reagent. After 15 minutes at room temperature, the solution is titrated with standardized 0.5 *N* sodium hydroxide in 90% methanol until, at an adjusted total volume of 150 ± 5 ml. (using 90% methanol as diluent), the hue matches that of the blank. The blank contains 100 ml. of hydroxylamine reagent plus 50 ml. of 90% methanol in a container of the type employed for the sample.

After a little practice, the analyst can so titrate the sample solution that it will be within 2 ml. of the end point when sufficient 90% methanol is added to bring the total volume to 150 ml.

The presence of active carbonyl compounds is evidenced by a shift of the thymol blue indicator toward red. At the end point, the red hue has just disappeared and the solution is orange in color. Careful color matching is necessary between sample and blank and is accomplished most easily against a white background. Didymium glasses may aid in making this comparison.

For aldehydes, the alkali consumed is equivalent, mole for mole, to the aldehyde in the sample.

For ketones, a correction factor is necessary. An average multiplier of 1.030 may be used for most routine work. For higher accuracy, the factor of the particular ketone must be determined as described below under discussion.

POTENTIOMETRIC TITRATION. The sample, containing up to 25 me. of aldehyde plus ketone, is weighed into a 400-ml. beaker containing 100 ml. of reagent. After 15 minutes at room temperature, the solution is titrated potentiometrically to a pH of 2.50. (Model G Beckman and Model 7661-A1 Leeds & Northrup pH meters equipped with standard glass and calomel electrodes were used for this work.) Sufficient 90% methanol is added to bring the total volume of sample solution to 150 ml. On dilution the apparent pH arises to about 2.65. The titration is continued dropwise until the pH equals that of a blank containing 100 ml. of reagent plus 50 ml. of 90% methanol. This diluted blank has an apparent pH of about 2.65.

Determination of Carbonyl Compounds in Presence of Free Acids or Bases. Mineral acids and organic acids of comparable ionization constants—e.g., sulfonic acids—will be titrated essentially quantitatively under the conditions of this analysis. Samples containing these types of acids may be titrated potentiometrically with 0.5 sodium hydroxide in 90% methanol to a pH of 2.50. Then the hydroxylamine hydrochloride reagent is added to the adjusted sample solution and the analysis is carried out in the usual way, using either the visual or potentiometric end point.

The acid correction also may be determined potentiometrically or visually on a separate sample.

Table I. Analytical Data for Aldehydes

No.	Substance	Source	Carbonyl Found, Weight %: Other method	Carbonyl Found, Weight %: 2.5 pH method	Acid Found, Weight %	Total
1	Formaldehyde	Merck, 36–38%	36.9[a]	(10)[b] 36.9 ± 0.2	..	....
2	Propionaldehyde	Eastman Kodak	95.3[c]	(4) 95.0 ± 0.2	..	....
3	Propionaldehyde	Redistilled	...	(4) 99.7 ± 0.2	0.2	99.9
4	Acrolein	.....	93.1[d]	(4) 93.3 ± 0.1	..	....
5	Glyceraldehyde	.....	...	(4) 100.5 ± 0.3	..	....
6	Butyraldehyde	Redistilled	...	(6) 99.9 ± 0.2	0.0	99.9
7	Isobutyraldehyde	Eastman Kodak	98.2[c]	(6) 98.3 ± 0.2	..	....
8	Methoxypropionaldehyde	Redistilled	...	(2) 100.1 ± 0.1	0.0	100.1
9	Isovaleraldehyde	Eastman Kodak	97.7[c]	(2) 97.7 ± 0.1	..	....
10	2-Methyl valeraldehyde	.....	95.8[c]	(2) 96.0 ± 0.2	..	....
11	Heptaldehyde	Eastman Kodak	96.4[e]	(2) 96.6 ± 0.2	..	....
12	2-Ethyl butyraldehyde	Carbide and Carbon	98.5[e]	(2) 98.7 ± 0.1	..	....
13	α-Ethyl-β-propyl acrolein	Eastman Kodak	99.8[c]	(4) 99.8 ± 0.3	..	....
14	3,5,5-Trimethylhexaldehyde	Du Pont	...	(8) 99.7 ± 0.2	..	....
15	Hexahydrobenzaldehyde	Distilled	...	(2) 99.6 ± 0.0	0.4	100.0
16	Benzaldehyde	Eastman Kodak	99.2[c]	(6) 99.2 ± 0.2	..	....
17	Benzaldehyde	Redistilled	...	(4) 99.6 ± 0.1	0.35	99.95
18	*m*-Nitrobenzaldehyde	Eastman Kodak	99.7[c]	(3) 99.6 ± 0.3	..	....
19	*p*-Nitrobenzaldehyde	Eastman Kodak	97.9[e]	(2) 97.8 ± 0.2	..	....
20	Tolualdehyde	Eastman Kodak	98.2[c]	(4) 98.5 ± 0.2	..	....
21	Salicylaldehyde	Redistilled	99.5[c]	(2) 99.6 ± 0.1	0.3	99.9
22	Vanillin	Eastman Kodak	99.6[e]	(4) 99.6 ± 0.1	..	....
23	Dimethylbenzaldehyde	Redistilled	...	(2) 100.0 ± 0.0	0.0	100.0
24	Trimethylbenzaldehyde	Redistilled	...	(2) 99.4 ± 0.1	0.3	99.7
25	Paraformaldehyde (trioxymethylene)	Mallinckrodt	93.2[a]	(1) 84.0[f] (2) 91.3 ± 0.2[g]	.. ..	....
26	Trioxane	Du Pont	...	(2) 0.0 ± 0.0 (2) 1.0 ± 0.0[h]	.. ..	....
27	Furfural	.....	98.2[c]	(2) 98.1 ± 0.3	..	....

[a] By Romijn iodometric procedure (*11*).
[b] Figures in parentheses represent number of individual determinations.
[c] Bryant-Smith method (*2*), 0.5 hour at room temperature.
[d] Bromination procedure (*9*).
[e] Bryant-Smith method (*2*), 2 hours at 100° C.
[f] Sample only partially soluble at room temperature.
[g] 1 hour at 100° C., sample soluble.
[h] 2 hours at 100° C.

The visual titration is made in the presence of a "blank" reagent—i.e., a solution containing no hydroxylamine hydrochloride. (The blank reagent is prepared by adding 5.5 ml. of 0.1% thymol blue to 840 ml. of 95% ethanol and diluting to 1 liter with water. Then hydrochloric acid is added until the color of a 100-ml. portion when diluted to 150 ml. with 90% methanol corresponds to the color of 100 ml. of the standard 2.5 pH hydroxylamine hydrochloride reagent plus 50 ml. of 90% methanol.) A weighed portion of the sample is added to 100 ml. of blank reagent and the resulting solution is titrated with 0.5 *N* sodium hydroxide in 90% methanol exactly as described in the general visual procedure for carbonyl compounds. The titer is corrected to represent the same weight of sample as that used for the separate carbonyl analysis and then subtracted directly from the total alkali consumed.

Only relatively minor corrections are required for most carboxylic acids. Samples containing acetic acid and others of about the same strength (ionization constant, $k = 5 \times 10^{-5}$) are corrected on the basis of 0.05 ml. of 0.5 *N* sodium hydroxide per gram of acid. This empirical correction is accurate for weak monobasic acids regardless of molecular weight. Stronger acids, such as formic and oxalic, lead to larger corrections. Each gram of formic acid, for example, consumes 0.5 ml. of 0.5 *N* base. For maximum accuracy, however, each acid should be checked individually in a known system containing a carbonyl compound which reacts quantitatively (see Table III). For systems containing only known carboxylic acids, a portion of the sample is titrated with standard caustic (sodium methylate in the presence of aldehydes, *6*) to the phenolphthalein end point. A second portion of the sample is treated directly with hydroxylamine hydrochloride. The resulting titer then is corrected for interference from the carboxylic acid by subtraction of its calculated titer to the carbonyl end point.

Samples containing free bases are best handled by adjusting to a pH of 2.50 prior to addition of the hydroxylamine reagent.

Determination of Acetals. The sample, containing up to 10 me. of acetal (no more than 20 me. of free plus combined carbonyl), is weighed into a 350-ml. Pyrex brand pressure bottle containing 100 ml. of hydroxylamine reagent. The bottle is stoppered securely and, together with a blank containing 100 ml. of reagent, is placed in a steam bath at 98° to 100° C. for 2 hours. (These Pyrex brand bottles have a standard beverage bottle–type opening. The most satisfactory closure is a Slip-Seal cap manufactured by the Slip-Seal Bottle Cap Company, 1750 California Ave., Long Beach, Calif., and distributed in the East by F. W. Krage Company, 327 Washington St., Newark, N. J. Other suitable closures include porcelain magnesia bottle stoppers or crimped bottle caps.) At the end of this time, the bottles are removed, cooled under running tap water, and titrated with standard 0.5 *N* sodium hydroxide in 90% methanol under the same conditions as those employed for free carbonyl titrations. (Occasionally these pressure bottles break, owing to thermal shock. It is recommended that a wire mesh basket be used to hold the bottles during the heat and cooling stages. The thymol blue indicator is not temperature-sensitive. Therefore, no special care is necessary in cooling the solutions.)

This procedure gives the total free plus combined carbonyl. When only acetal is desired, the free carbonyl, as determined by separate analysis, is subtracted from the total carbonyl value. If no more than 1 me. of free carbonyl is present, the sample solution can be adjusted before heating. The sample plus reagent in the pressure bottle is allowed to stand 15 minutes at room temperature and then titrated with 0.5 *N* sodium hydroxide in 90% methanol until the hue matches that of the undiluted blank. Then the solution is stoppered and heated as indicated above.

ANALYTICAL RESULTS

Analyses of a variety of aldehydes are recorded in Table I. All results are calculated on the basis of Reaction 1.

The accuracy of the method for the types of aldehydes listed in Table I is indicated by comparison of the results by the new procedure with those of other standard methods and by the totals of aldehyde plus acid given by the laboratory-distilled materials. In general, precision and accuracy, based on maximum deviation, are within ±0.1%. Paraformaldehyde tended to give low results. At room temperature this sample was not completely soluble in the hydroxylamine reagent. At 100° C., the solution became clear after about 20 minutes. Trioxane, the cyclic trimer of formaldehyde, proved extremely resistant to the reagent. After 2 hours at 100° C., only 1% reaction had occurred. Walker reported that a 20% solution of trioxane in 6% sulfuric acid was only 50% hydrolyzed after 6 hours of refluxing. Apparently a nearly anhydrous system and strong acids are necessary for fairly rapid depolymerization (*16*).

In Table II are presented analytical data for a variety of ketones. Because all common ketoximes are slightly basic at the pH of the end point, a correction factor is necessary. The factor employed for each ketone which reacted essentially quantitatively is included in this table. In many cases the factor was based on hydrochloric acid recovery in the presence of the appropriate oxime (see Discussion).

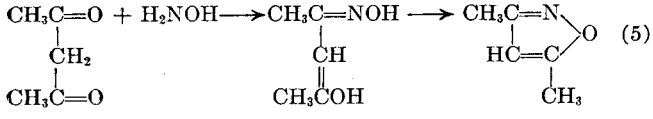
Most of the methyl ketones examined reacted quantitatively with the hydroxylamine reagent. However, methyl *tert*-butyl (pinacolone), methyl hexyl, and methyl 2-naphthyl ketones required 1 hour at room temperature and acetophenone required 2 hours at 100° C. before reaction was complete.

Cyclopentanone and cyclohexanone usually reacted quantitatively. Occasionally, however, results on essentially pure

samples tended to analyze about 1% low as compared to other methods. The reasons for these discrepancies are not known, but are being studied further.

Although diethyl and ethyl isopropyl ketones appeared to react completely in 15 minutes at room temperature, other ethyl ketones studied required 1 hour or more. Higher ketones indicated lower yields which might be due to slower rates of reaction or to unfavorable equilibria.

Diacetyl ($CH_3COCOCH_3$) reacted essentially quantitatively at room temperature. Acetylacetone ($CH_3COCH_2COCH_3$) and probably other 2-diketones gave a net value about equivalent to complete reaction of one carbonyl group. Probably cyclization occurred to give dimethyl isoxazole (*10*), even though excess hydroxylamine was present.



(5)

The presence of 2-diketones in unknown samples probably can be verified by the copper precipitation method of Seaman, Woods, and Massad (*12*). The net reaction of acetonylacetone (2,5-hexanedione, $CH_3COCH_2CH_2COCH_3$) tended to decrease with time. On heating, the solution turned dark brown in color. This behavior appears to merit further study.

The effects of carboxylic acids on analyses for some aldehydes and ketones are indicated in Table III. In most of the experimental work, cyclohexanone was employed as the carbonyl compound.

These results demonstrate that up to 10 grams of weak organic acids introduce errors no greater than +0.4%. When corrected, the data indicated an average accuracy of ±0.1% absolute. After correction, samples containing nearly 20 grams of acetic acid gave results accurate to 0.1% absolute. Formic acid interfered markedly but after the experimental correction factor was applied, the net results were within 0.2% absolute. A similar correction would be expected for samples containing oxalic acid.

Analytical results on several acetals are given in Table IV. With the exception of the distilled materials, the samples were C.P. grade and were analyzed as received. In all cases the samples were weighed into the hydroxylamine reagent and allowed to stand 0.5 hour at room temperature. With the one exception noted below, those solutions in which reaction had taken place were titrated with 0.5 *N* sodium hydroxide without dilution with 90% methanol and then heated 2 hours at 100° C.

Acetal (acetaldehyde diethyl acetal) proved to be considerably less stable to hydrolysis than the other acetals studied. This compound appeared to react completely at room temperature. No further reaction was observed after heating. When the same acetal sample was heated directly, the results checked those formed at room temperature. Acetal is the only acetal studied in this laboratory which was hydrolyzed completely in 2 hours at 100° C. by the pyridine–bromophenol blue method of Bryant and Smith (*2*). With this reagent no reaction occurred at room temperature.

Two of the three cyclic acetals reported at the bottom of Table IV were resistant to hydrolysis under conditions of the present method. However, although 1,3-dioxolane ($H_2COCH_2CH_2O$) was inert, hydroxymethyl dioxolane ($HOCH_2CHOCH_2CH_2O$) appeared to react essentially quantitatively.

Table II. Analytical Data for Ketones

No.	Ketone	Source	Carbonyl Found, Weight %: Other method	2.5 pH method	Factor
1	Acetone	Merck	99.6[a]	(10)[b] 99.6 ± 0.2	1.030[c]
2	Methyl ethyl	Eastman Kodak	98.2	(4) 98.3 ± 0.1	1.024[c]
			96.5	(4) 96.6 ± 0.2	1.024
3	Diethyl	.....	98.6	(8) 98.6 ± 0.2	1.024[c]
4	Methyl propyl	Eastman Kodak	96.3	(2) 96.2 ± 0.0	1.030
5	Methyl isopropyl	Eastman Kodak	96.5	(3) 96.5 ± 0.3	1.024
6	Methyl isobutyl	Eastman Kodak	96.9	(2) 97.2 ± 0.2	1.024
7	Methyl *tert*-butyl	Eastman Kodak	96.0	(2) 94.6 ± 0.5	1.030[c]
				(4) 96.1 ± 0.1[d]	1.030
8	Ethyl isopropyl	P. B. Kletzke	44.3	(2) 44.4 ± 0.3	1.024
9	Methyl amyl	P. B. Kletzke	95.2	(2) 95.3 ± 0.1	1.024[c]
10	Ethyl butyl	P. B. Kletzke	89.5	(2) 87.4 ± 0.4	1.024[c]
				(2) 89.8 ± 0.3[d]	1.024
11	Diisopropyl	P. B. Kletzke	106.2	34.5	...
				(2) 40.5 ± 0.5[e]	...
12	Methyl hexyl	Redistilled	...	(2) 92.8 ± 0.1	1.030[c]
				(3) 99.8 ± 0.1[d]	1.030
13	Ethyl amyl	P. B. Kletzke	82.0	(2) 73.3 ± 0.3	1.024
				(2) 79.6 ± 0.2[d]	1.024
14	Propyl butyl	P. B. Kletzke	98.8	(2) 91.8 ± 0.4	1.024[c]
				(2) 94.6 ± 0.2[d]	1.024
15	Propyl amyl	P. B. Kletzke	62.3	(2) 56.2 ± 0.3	1.024
				(2) 57.6 ± 0.3[d]	1.024
16	Methyl undecyl	Eastman Kodak	99.8	(2) 99.5 ± 0.0	1.030
17	Ethyl undecyl	Eastman Kodak	100.8	(2) 98.3 ± 0.0	1.030
				(2) 100.4 ± 0.3[d]	1.030
18	Cyclopentanone	.....	98.5	(2) 97.0 ± 0.1	1.032[c]
			98.7	(8) 98.8 ± 0.2	1.032
19	Cyclohexanone	Du Pont	99.5[f]	(6) 98.8 ± 0.4	1.032[c]
			98.7	(6) 98.8 ± 0.2	1.032
			96.7	(6) 96.6 ± 0.2	1.032
20	2-Hydroxymethyl cyclohexanone	.....	...	(2) 100.3 ± 0.1	1.030
21	Acetophenone	Eastman Kodak	98.0	(2) 89.8 ± 0.2	1.030[c]
			99.9	(4) 99.7 ± 0.1[g]	1.030
22	Benzophenone	Eastman Kodak	98.8	10	...
				70[g]	...
23	Benzoin	Eastman Kodak	99.4	36	...
				86[g]	...
24	Methyl naphthyl	Eastman Kodak	99.1	(2) 85.0 ± 0.2	1.015[c]
				(2) 99.8 ± 0.0[d]	1.015
25	Diacetyl	Eastman Kodak	94.8	(2) 94.1 ± 0.2	1.030
				(1) 94.2[d]	1.030
				(2) 94.6 ± 0.1[g]	1.030
26	Acetylacetone	Eastman Kodak	95.5[h]	(6) 50.5 ± 0.3	...
				(2) 50.2 ± 0.3[i]	...
				(2) 49.3 ± 0.3[j]	...
27	Acetonyl acetone	Eastman Kodak	94.8	(9) 98.6 ± 0.2	1.030
				(2) 95.3 ± 0.2[d]	1.030
				(4) 46.0 ± 2[g]	...
28	Benzil	Eastman Kodak	109.5[k]	(2) 70.2 ± 0.0	...

[a] Bryant-Smith method (*2*), 0.5 hour at room temperature. Unless otherwise noted, all other determinations in this column were made by this method using 2 hours at 100° C.
[b] Figures in parentheses represent number of individual determinations.
[c] Factor determined experimentally; all others based on comparison with similar compounds or with results obtained by an independent method.
[d] 1 hour at room temperature.
[e] 2 hours at 100° C.
[f] Karl Fischer procedure (*8*).
[g] 1 hour at 100° C.
[h] Bryant-Smith method (*2*), 2 days at room temperature.
[i] 6 hours at room temperature.
[j] 18 hours at room temperature.
[k] Calculated on basis of one carbonyl group.

INTERFERING SUBSTANCES

From an examination of Table II it is evident that higher ketones tend to react incompletely. In general, a partial but reproducible reaction may be expected. Most hydrocarbons, although inert, are insoluble in the reagent. Often the addition of 90% methanol before titration tends to render the system homogeneous.

Alcohols, ethers, and esters generally are inert. Cyclohexenyl cyclohexyl ether, however, is hydrolyzed appreciably at room temperature and quantitatively in 0.5

Table III. Analytical Data for Carbonyl Compounds in Presence of Carboxylic Acids

Carbonyl Compound, Weight %	Acid, Weight %	Sample Weight, Grams	Carbonyl Found, Weight % Uncorrected	Corrected[a]
Valeraldehyde	Valeric			
8.5	91.5	15.2	8.8	8.5
33.6	66.4	7.0	33.7	33.5
Benzaldehyde	Benzoic			
88.8	11.2	2.2	88.6, 88.7	..
Acetone	Oleic			
24.2	75.8	6.6	24.3	24.0
Cyclohexanone	Formic			
25.7	74.3	5.5	27.8	25.8[b]
43.2	56.8	4.4	44.9	43.4[b]
Cyclohexanone	Acetic			
0.3	99.7	20.8	0.7	0.20, 0.20
0.5	99.5	20.8	0.9	0.40, 0.45
0.7	99.3	20.8	1.1	0.60, 0.60
21.3	78.7	8.9	21.5	21.2
24.0	76.0	7.9	24.2	24.0
27.5	72.5	6.9	27.8	27.6
32.2	67.8	5.9	32.4	32.2
38.8	61.2	4.9	39.0	38.8
48.7	51.3	3.9	48.8	48.6
65.5	34.5	2.9	65.5	65.3
Cyclohexanone	Propionic			
24.7	75.3	7.7	24.8	24.6
Cyclohexanone	Caproic			
30.0	70.0	6.3	30.1	29.9
Cyclohexanone	Succinic			
27.5	72.5	6.9	27.7	27.5
Cyclohexanone	Adipic			
16.0	84.0	11.9	16.4	16.0
21.3	78.7	8.9	21.5	21.2
27.5	72.5	6.9	27.7	27.5
Cyclohexanone	Benzoic			
27.5	72.5	6.9	27.7	27.5

[a] Except where noted, empirical correction of 0.05 ml. of 0.5 *N* NaOH per gram of acid was applied.
[b] Correction for formic acid was 0.5 ml. 0.5 *N* NaOH per gram of acid.

Table IV. Analytical Data for Acetals

Substance	Source	Carbonyl Found, Weight % Free	Combined
Methylal	Distilled[a]	0.0	(10)[b] 92.2 ± 0.2
Acetal	Eastman Kodak	99.3 ± 0.3	(2) 0.0 ± 0.1
Ethylal	Eastman Kodak	0.2	(2) 97.2 ± 0.1
Propylal	Eastman Kodak	0.9	(2) 92.2 ± 0.2
Trimethoxyethane	Distilled	0.0	(2) 101.0 ± 0.2
Triethoxyethane	Distilled	0.0	(4) 100.8 ± 0.8
Tributoxyethane	Distilled	6.0	(2) 91.7 ± 0.4
Dimethylacetal	Eastman Kodak	0.0	(2) 97.4 ± 0.2
Methoxymethylal	Eastman Kodak	0.0	(2) 98.1 ± 0.3
Methoxymethoxy-ethanol[c]	Du Pont	0.0	(6) 99.3 ± 0.4
Dinonoxynonane	Distilled	0.0	(2) 100.3 ± 0.0
1,3-Dioxolane	Du Pont	0.0	(2) 0.0 ± 0.0
1,3-Dioxane[d]	Du Pont	0.0	(2) 10.0 ± 0.5
5-Hydroxymethyl-1,3-dioxolane	Du Pont	0.0	(2) 99.5 ± 0.2

[a] Methylal-methanol azeotrope contains 7.8% methanol.
[b] Figures in parentheses represent number of individual determinations.
[c] Mixed formal of methanol, formaldehyde, and glycol—i.e., $CH_3OCH_2OCH_2CH_2OH$.
[d] Made from trimethylene glycol and formaldehyde.

hour at 60° C. Vinyl acetate (and probably vinyl ethers) is subject to hydrolysis, as shown in Table V.

Water in large quantities changes the properties of the environment. Up to 10 grams of water will not interfere appreciably, provided a comparable amount is added to the blank. A sample of cyclohexanone analyzing 94.8% gave a value of 94.7% after addition of 10 ml. of water to both sample and blank.

Anhydrides probably react with hydroxylamine to form the corresponding hydroxamic acids. For example, under the standard conditions (15 minutes at room temperature) weighed quantities of propionic anhydride gave the following results:

Propionic Anhydride G.	Propionic Anhydride MM.	NaOH Consumed Ml.	NaOH Consumed MM.
0.05	0.40	0.35	0.18
0.1	0.75	0.70	0.35
0.2	1.55	1.40	0.70
0.3	2.30	2.10	1.05
0.4	3.10	2.85	1.43
0.5	3.85	3.45	1.73

Based on these data, the millimoles of alkali required were equivalent to about half the millimoles of anhydride present. Normally the alkali consumption would be expected to be twice this value. Therefore, empirical corrections for anhydrides probably are necessary.

Peroxides appear to interfere, judged by the effects of benzoyl peroxide on the determination of cyclohexanone.

Three samples containing 9 millimoles of cyclohexanone and 0.1 millimole of peroxide were added to hydroxylamine reagent and allowed to stand at room temperature for 0.5 hour, at 60° C. for 0.5 hour, and at 100° C. for 0.5 hour. Then the solutions were titrated with standard sodium hydroxide. After correction for the cyclohexanone contribution, about 2 millimoles of alkali were consumed per millimole of benzoyl peroxide in the samples which stood at room temperature and at 60° C., while about 4 millimoles of alkali were used up per millimole of peroxide by the 100° C. samples.

Carboxylic acids do not appear to interfere seriously in the determination of acetals. Ten-millimole samples of methylal and propylal containing increasing quantities of acetic acid were added to the hydroxylamine reagent and heated 2 hours at 100° C. After correction of the alkali titer for acetic acid interference (0.05 ml. of 0.5 *N* sodium hydroxide per gram), the following results were obtained:

Acetic Acid, Ml.	Weight % Acetal Recovered Methylal	Propylal
0	100.0	100.0
1	99.7	100.0
5	99.0	101.0
10	99.0	99.0

Acrolein, and probably other aldehydes having active unsaturated carbon to carbon bonds, apparently tend to add hydrochloric acid when the reagent and the aldehyde are heated. Results were about 15% low after 1 hour at 100° C. No interference was observed, however, after acrolein and the reagent had stood for periods of up to 2 hours at room temperature.

DISCUSSION

The present method was developed to meet the specific need for a rapid procedure for the determination of cyclohexanone in the presence of large amounts of organic acids. All previous acidimetric methods employed indicators in the range above pH 4, where carboxylic acid interference was appreciable. Therefore, initial studies were directed toward the choice of an indicator which would register a visual change at a pH where carboxylic acids were scarcely titrated. At the thymol blue end point (pH 2.5 to 2.8) hydrochloric acid was almost completely neutralized, whereas acetic acid was only slightly titrated. The end point was considerably sharper in a strongly alcoholic environment. pH 2.5 was below the equivalence point (ca. pH 3.2) of hydroxylamine hydrochloride solution.

In order to demonstrate that hydrochloric acid added to the hydroxylamine solution at an apparent pH of 2.50 could be titrated quantitatively, known quantities of this acid were added to 100-ml. volumes of reagent. The solutions were titrated with 0.5 *N* sodium hydroxide in 90% methanol. In all cases 99.8 to 99.9% of the added acid was recovered. In the presence of 2.75 grams of cyclohexanone oxime, however, recoveries of added hydrochloric acid were only 96.5 to 97.0%. (When available, the oximes were obtained from Eastman Kodak Company. The others studied were prepared in the laboratory. In all cases the oximes were recrystallized from water and dried before use.) Two grams of butyraldoxime, on the other hand, had no effect on acid recovery. These observations suggested that the ketoxime was mildly basic at apparent pH 2.5, whereas the aldoxime was essentially neutral. Selected ketoximes and aldoximes in glacial acetic acid were titrated with perchloric acid in glacial acetic acid solution, an accepted reagent for the titration of weak bases (*14, 15*). The oximes of cyclohexanone, methyl isopropyl ketone, and acetone gave reasonably sharp potentiometric breaks, whereas those of butyraldehyde and heptaldehyde were too weak to be titrated.

A correction factor of 1.032 for cyclohexanone was established from the average of hydrochloric acid recovery data in the presence of its oxime. Further confirmation of this factor was

Table V. Hydrolysis of Vinyl Acetate in 2.5 pH Reagent

Sample Weight, Grams	Condition		Weight % Reaction
	Hours	° C.	
1.6	0.5	Room temp.	0.5
4.0	0.5	Room temp.	0.6
1.6	18	Room temp.	26.0
0.5	1.5	100	93.7
1.0	1.5	100	96.0
2.0	1.5	100	94.2
1.0	2	100	95.7, 96.2

established by direct analysis of carefully distilled cyclohexanone. This sample analyzed 99.6 ± 0.2% by the Karl Fischer procedure (*8*) and 99.7 ± 0.4% by the pyridine–bromophenol blue method (*2*). (A total of 0.4% cyclohexanol plus water was found by analysis. The results on this sample are not included in Table II.) Analyses of thirteen individual samples gave an average correction factor to 99.6% of 1.032 with a deviation of ±0.003. Similar experiments were made in establishing the correction factors to be used in the determination of the other ketones as noted in Table II. In general, acid recovery in the presence of purified oxime was considered the more reliable means for establishing these factors, because the values obtained were not dependent on purity of the ketone as determined by another method.

SEMIMICRO APPLICATIONS OF THE 2.5 pH REAGENT

The present visual method was adapted readily to a semimicro scale.

The sample, containing up to 1 me. of free carbonyl compound, is weighed into a 50-ml. glass-stoppered borosilicate glass bottle containing 20 ml. of the hydroxylamine reagent. After 15 minutes at room temperature, the solution is titrated with 0.1 *N* sodium hydroxide in 90% methanol until at an adjusted total volume of 35 ml. the hue of the sample matches that of a blank containing 20 ml. of hydroxylamine reagent plus 15 ml. of 90% methanol.

All the compounds listed in Tables I and II which reacted quantitatively gave results of comparable precision and accuracy by the semimicroprocedure.

For the analysis of acetals, special 50-ml. heavy-walled Pyrex brand bottles are used which employ the same caps as the 350-ml. Pyrex brand bottles.

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Analysis and Characterization of Pure Compounds and Mixtures of Compounds

Solubility Procedure

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THE solubility of a pure solid compound in any given solvent at any given temperature is as characteristic of the compound as other physical properties such as the melting point or boiling point. However, the measurement of solubility characteristics to establish the identity of compounds or to serve as a criterion of their purity has not come into general use, except where other methods completely fail. This is probably because of the relatively large amounts of material required, the tediousness of carrying out a series of precise solubility determinations, and the difficulty of expressing the results in as simple a form as is done with melting points and boiling points.

A critical review of three analytical methods based on solubility procedures is given by Bennett (*2*) and one of these, the Northrop and Kunitz procedure, is also reviewed by Herriott (*7*). All the procedures described in the literature have been carried out at essentially one temperature. The idea of having fixed amounts of solute and solvent and using the measurement of the temperature of complete solution as a measure of a given component has not been applied to the analysis of solids, although in the case of liquid mixtures, various analytical procedures for the determination of a given component have been based on the temperature at which the single-phase system changes to a two-phase system (*8*).

Where conventional melting points and mixed melting points fail to prove the identity of two materials or to serve as a criterion of purity, owing to decomposition, the procedure here described based on the temperature of complete solution may often be substituted. Likewise, where thermal analysis of binary mix-

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Table I. Solubility Temperatures of Amino Acids in Water

Amino Acid	Observed Sol. Temp., ° C.[a]	Calcd. Sol. Temp., ° C. from Literature Data[a,b]	Reference	Depression of Sol. Temp. Due to 1% Soluble Impurity[c], ° C.
Glycine	.....	6.7 ± 0.3 at 6:1	(3)	0.42
DL-Threonine	14.0 at 6:1	.....	..	0.70
L-Histidine.HCl.H_2O	23.5 at 6:1	.....	..	...
DL-Alanine	26.3 at 6:1	24.8 ± 0.3 at 6:1	(3)	0.77
L-Alanine	.....	25.1 ± 0.2 at 6:1	(3)	0.93
Taurine	.....	39.8 ± 0.2 at 6:1	(4)	0.35
DL-Allothreonine	47.6 at 6:1	.....	..	0.63
L-Asparagine.H_2O	.....	65.2 ± 0.2 at 6:1	(4)	0.26
DL-Serine	.....	68.9 ± 0.2 at 6:1	(4)	0.42
DL-Glutamic acid	.....	84.7 ± 0.6 at 6:1	(3)	0.28
DL-Valine	94.5 at 6:1	92.8 ± 0.2 at 6:1	(3)	0.60
DL-Methionone	.....	97.0 ± 0.2 at 6:1	(4)	0.49
L-Isoleucine	.....	18.7 ± 2.1 at 25:1	(4)	2.50
L-Phenylalanine	.....	43.6 ± 0.5 at 25:1	(4)	0.62
L-Glutamic acid	.....	66.3 ± 0.3 at 25:1	(3)	0.27
DL-Isoleucine	.....	67.2 ± 0.5 at 25:1	(3)	0.57
DL-Phenylalanine	81.5 at 25:1	78.3 ± 0.3 at 25:1	(3)	0.43
DL-Aspartic acid	70.8 at 25:1	71.4 ± 0.2 at 25:1	(3)	0.33
L-Aspartic acid	.....	84.5 ± 0.4 at 25:1	(3)	0.29
DL-Norleucine	.....	89.5 ± 0.4 at 25:1	(3)	0.41
L-Tryptophan	.....	90.0 ± 0.4 at 25:1	(4)	0.42
DL-Leucine	Approx. 105.5 at 25:1	98.0 ± 0.2 at 25:1	(3)	0.37

[a] Water to amino acid ratio = 6:1 or 25:1 as indicated.
[b] ± values are two thirds of mean deviation.
[c] Calculated from expression $\log S = a + bt + ct^2$ by differentiation and substitution of appropriate constants.

tures by means of a melting point curve is not feasible, the substitution of a similar type of curve, based on the temperature of complete solution as determined by this solubility procedure, should frequently be satisfactory.

This paper describes a convenient method of determining the temperature at which a solute will dissolve in a fixed amount of solvent, and demonstrates how this temperature, designated as the "solubility temperature," can be of use in the characterization of compounds, in the determination of their purity, and in the analysis of mixtures.

The most important new feature of this analytical technique is the use of the temperature of complete solution in a manner analogous to the way melting point data for pure compounds and mixtures have been previously handled. Also of importance is the development of a standardized procedure for accurately and conveniently determining the solubility of relatively small amounts of a substance in a solvent over a wide temperature range.

The method requires 50 to 200 mg. of material; however, all can be recovered after the determination is finished, or some other analysis can be carried out with the accurately weighed sample. Less than an hour is required for a determination.

All the work described in this paper has been done with amino acids using water as the solvent, but there are no apparent reasons why the method should not be equally applicable, with water or other solvents, to other classes of compounds.

PROCEDURE

The finely ground solid is accurately weighed into a 15 × 75 mm. borosilicate glass test tube; care is taken that no solid adheres to the walls of the tube. The weight of sample is such that the final volume will approximate 1 ml. after dilution with the solvent. A weight of solvent, usually 3, 6, or 25 times that of the sample, is introduced from a calibrated 2-ml. Koch microburet with the necessary corrections for thermal expansion. The addition should be so conducted that no liquid touches the side of the test tube within 5 cm. of the top. The tube is immediately and rapidly sealed, using a small pointed gas-oxygen flame. This is done by first sealing a rod on the lip of the test tube while holding the test tube in a vertical plane at about a 60° angle to the gas-oxygen flame. The test tube is heated intensely with a small pointed flame about 2 cm. below the lip while rotating, and the heated portion is then drawn out to a fine capillary. After 5 seconds, the capillary is sealed off as close to the test tube as possible, so that there will be no capillary tube where small amounts of the solid can become lodged.

The temperature of complete solution is determined by attaching one or more tubes with rubber bands to the end of a shaft which can be rotated at 60 to 100 r.p.m. and which is mounted 30° from the horizontal so that the end can dip into a 7-liter battery jar filled with water. The tubes, attached at right angles to the shaft, can be immersed 7.5 cm. (3 inches) below the liquid level and still rotate only 30° from the vertical. This furnishes good agitation, for the contents of the tube fall from one end to the other as it is rotated. A strong light is placed so as to illuminate the tubes. The temperature of the bath is controlled by an internal electrical heater connected through a voltage regulator. The bath is stirred vigorously to prevent any appreciable temperature differentials. Temperatures are measured by a calibrated 0° to 100° partial immersion thermometer graduated in 0.1° (Kimble Glass No. 43606).

As the tube is rotated, the temperature of the bath is rapidly raised to within 2° or 3° of the solubility temperature. This point is easily recognized because most of the crystals have disappeared. The rate of heating is then reduced to 0.1° per minute. As complete solution is approached, the rotation of the tube is stopped for short intervals, so that the contents may be more closely examined. These short intervals should not amount to more than 30 seconds every 2 minutes. The temperature at which the last crystal dissolves is taken as the solubility temperature. For tubes prepared from the same sample, this temperature is reproducible to 0.2° C. For phenylalanine, valine, aspartic acid, and other amino acids which are not wet too well by water, and which crystallize rapidly when their hot solutions are cooled, more reproducible results are obtained by heating the sample tube rapidly to complete solution, then removing the shaft with attached tube from the bath, and rotating 10 minutes in the air with the shaft in a horizontal position. During this period, crystals will form in the sample tube and the temperature of the water bath will fall rapidly (if the front part of the cover is removed) to a few degrees below the expected solubility temperatures. After 10 minutes, the shaft and tube are replaced in the water bath and the solubility temperature is determined in the usual manner.

APPLICATION TO AMINO ACIDS

Of the important amino acids, four have such small solubilities in water that excessive amounts of water are needed to dissolve them: cystine, tyrosine, diiodotyrosine, and thyroxine. Four others—L-arginine hydrochloride, DL-lysine hydrochloride, proline, and hydroxyproline—are extremely soluble in water. Most of the remaining amino acids fall into two groups. One group has solubility temperatures ranging from 6.7° to 97° if a ratio of 6 parts of water to 1 of amino acid is used. The other group has solubility temperatures covering a somewhat similar range if a ratio of 25 parts of water to 1 of amino acid is used. The solubility temperatures for the amino acids in these two groups have been calculated from data in the literature. New data are presented for DL-threonine and L-histidine monohydrochloride. The solubility temperatures of four carefully purified amino acids—DL-alanine, DL-aspartic acid, DL-phenylalanine, and DL-valine—have also been determined in order to compare these values with the solubility temperatures calculated from the literature solubility data. With the exception of DL-phenylalanine, the solubility temperatures for these six amino acids are believed to be accurate to approximately 0.2° C., because the amino acids were recrystallized until their solubility temperatures remained constant.

A comparison of the experimentally determined solubility temperatures with the calculated values indicates that the experimentally determined values are often a few degrees higher than the calculated values. In one case (DL-leucine) the difference is 7°, but, because the calculated solubility temperatures for all the amino acids are based on data obtained below 60° C., this is due to the large extrapolation of the literature data. For the amino acids having solubility temperatures below 60° C., the temperature differential is believed to be due chiefly to the fact that the experimental values are obtained in a dynamic system, whereas the calculated values are based on data obtained under equilibrium conditions.

The temperature at which a solid dissolves in a given amount of solvent is a characteristic property of the solid, and has been designated the "solubility temperature" when it is determined under the described conditions. Like melting points, solubility temperatures can be used to characterize compounds, as a criteria of purity, and to analyze mixtures. They are especially useful with solids which have unsatisfactory melting points due to decomposition. A convenient procedure for rapidly and accurately determining solubility temperatures is described, and their usefulness in the characterization and analysis of amino acids is illustrated.

The solubility of L-valine changes relatively little with temperature and is such that it falls between the two groups given in Table I. In addition, the literature solubility data indicate that the mode of crystallization influences the solubility (*5*). The more stable form should have a solubility temperature of approximately 37° with a water to L-valine ratio of 12 to 1 (data from Figure 1 of *5*).

It is suggested that future workers determine solubility temperatures with water to amino acid ratios of 3, 6, 12, 25, or 50 or multiples thereof, so that data from different laboratories will be on a comparable basis.

ERRORS AND REPRODUCIBILITY

Inasmuch as the temperature is being slowly raised during the course of a solubility temperature determination, the temperature of complete solution determined in this way must be somewhat higher than the corresponding temperature determined under equilibrium conditions. In the determination of melting points, there is a similar difference between the "melting point" determined by the common capillary tube method, and the true melting point determined under equilibrium conditions. With DL-threonine and DL-allothreonine, on which much of the pioneering work was done, the difference appears to be only a fraction of a degree. With other amino acids, this difference may amount to more than a degree, but it will be a constant under any given set of experimental conditions for any particular amino acid. The extent of this temperature differential depends on three factors, among others: the size of the crystals dissolving, the ease with which the crystals are wetted by the solvent, and the rate at which the temperature of the bath is raised.

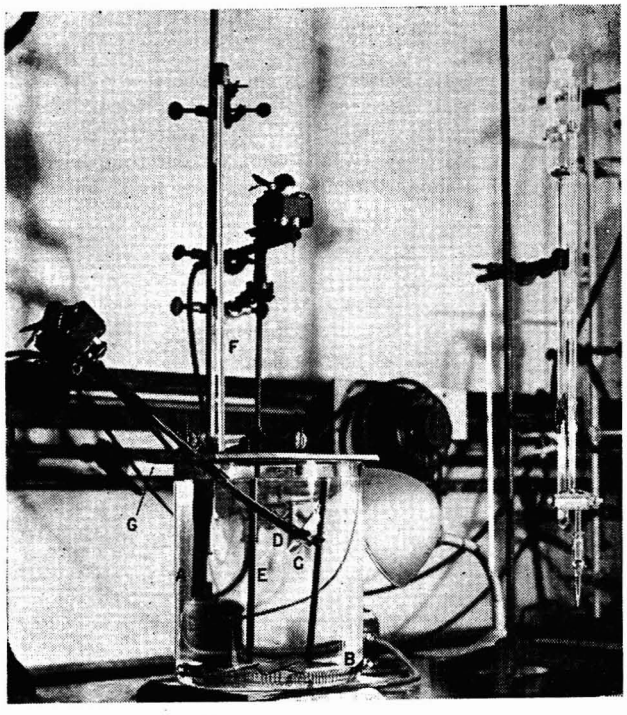

Figure 1. Apparatus

A. Baffle
B. Heater
C. Sample tube with sample
D. Shaft on which sample is mounted
E. Stirrer
F. Thermometer graduated in 0.1° C.
G. Transite cover (front part is removable)

The crystal size can be controlled by grinding the sample to a fine powder, or by dissolving the sample in the sealed tube and then cooling to obtain some small crystals. Errors due to the fact that crystals are difficultly wet by the solvent might be minimized by using a wetting agent, but it is preferable to use a pure solvent and to accept this small temperature differential. If the temperature of the bath is raised at a more rapid rate than 0.1° C. per minute, the solubility temperature will be somewhat higher than under the specified conditions.

With the more soluble amino acids, such as threonine and allothreonine, the rate of solution of the amino acids is surprisingly rapid. Thus a finely powdered threonine-allothreonine sample, having a certain solubility temperature, could be obtained in the form of large crystals by allowing the sealed tube to stand for several days after the completion of the run. When the solubility temperature was redetermined, a value only about 1° high was obtained, in spite of the fact that the surface area of the crystals exposed to the solvent had been reduced by many thousandfold. With the less soluble amino acids, especially those not wet well by water, crystal size is much more important and should be as small as possible. Thus in the case of DL-phenylalanine, high results will be obtained unless the sample is first dissolved and the solubility temperature determined on the freshly formed crystals. Even when this procedure is followed with this amino acid, the results are less accurate than in the case of the other amino acids, and occasionally results 1° high will be obtained.

The somewhat empirical nature of solubility temperatures is apparent from the above discussion. The small differences between solubility temperatures obtained by the described procedure and the true temperatures of complete solution, determined under static, ideal conditions, are due to several factors which will vary from compound to compound.

The precision obtained in the determination of solubility temperatures depends upon the accuracy with which the solvent is added to the sample, upon sealing the tube without loss of either component, and upon the accuracy with which the end point is observed. It is possible to add the solvent with an accuracy of 1 part per thousand when a calibrated microburet is used and allowance is made for the temperature of the solvent in the buret. This has been checked by calculating the weight of the water in the sealed-off tube from the weights of the two parts of the sealed-off tube, and comparing this with the volume of water added. Care must be taken that the sample and the solvent are deposited in the bottom of the test tube and not on the walls, where they would be destroyed or volatilized when the tube is sealed off. When the tube is sealed, the position in which it is held must be such that the flame does not enter it. In some

cases it may be advisable to cool the solvent before sealing off the tube in order to minimize losses; this has not been done in the present work.

The temperature of complete solution can be observed within 0.2°, providing the solution is such that the last crystals are clearly visible and no insoluble materials are present. If more than a trace of insoluble material is present, it is necessary to try to estimate the temperature at which no more material dissolves. This, of course, is difficult and inaccurate.

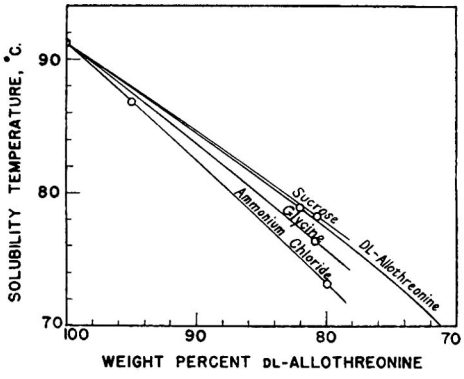

Figure 2. Variation of Solubility Temperature of DL-Allothreonine in Presence of Various Compounds

Water-solid ratio of 3:1. DL-Allothreonine curve is for DL-allothreonine alone; in this case, % DL-allothreonine used relative to amount required for 3:1 ratio is plotted as abscissa. This gives a curve corresponding to a soluble inert impurity

The reproducibility of solubility temperatures is illustrated by the values obtained for DL-allothreonine during its purification by an extended series of recrystallizations. A ratio of 10 parts of water to 3 of amino acid was used. Values found were: crude, 80.7° C.; first crystallization, 81.3°; second recrystallization, 82.5°; fourth recrystallization, 82.7°; sixth recrystallization, 83.4°; eighth recrystallization, 83.5°.

EFFECT OF ADDED COMPONENTS

The solubility temperature will be depressed by the presence of soluble impurities because each component of a solid phase dissolves more or less independently of the others, and there is less of the main material to dissolve in the fixed amount of solvent. If the soluble impurity is inert in the sense that it has no effect on the solubility of the main material, the lowering of the solubility temperature can be easily calculated from the temperature-solubility data of the compound in question. In practice, soluble impurities will often affect the solubility of the main component either positively or negatively.

Solubility temperatures for DL-allothreonine in the presence of ammonium chloride, sucrose, and glycine are given in Figure 2. The effect of a soluble inert impurity is given by the DL-allothreonine curve, in which the weight of DL-allothreonine used per volume of solvent is the same as the weight of the DL-allothreonine component in the two component mixtures—that is, no second component was used and the weight of the water equaled: (300) × (grams of DL-allothreonine)/(weight % DL-allothreonine plotted as abscissa). Sucrose is without effect, glycine lowers the solubility temperature somewhat, and ammonium chloride lowers it even more. Even with ammonium chloride, however, the error introduced is not excessive when less than 5% is present. In the presence of larger amounts of a material acting as ammonium chloride does in this case, it would be necessary to construct a solubility temperature–composition diagram as described herewith.

The identity of two solid materials can be established by determining their mixed solubility temperatures; if there is no depression, the two are identical, providing they do not pass through the liquid state before dissolving. In the case of liquids, the solubility temperature procedure may fail. It has been found by Ivan Christoffel of these laboratories that at least one pair of liquid isomers—2-phenyl-2-methoxyethanol and 1-phenyl-2-methoxyethanol—have nearly identical solubility temperatures and the solubility temperatures of mixtures of these show no depression.

APPLICATION TO ANALYSIS OF MIXTURES

A binary mixture can be analyzed by comparing its solubility temperature with that of known mixtures of the two pure compounds. The solubility temperature–composition diagram of the DL-threonine–DL-allothreonine system is typical (Figure 3).

The solubility temperature of DL-allothreonine is 91.2° C.; mixtures of DL-allothreonine with DL-threonine will have lower solubility temperatures until a composition corresponding to 37% DL-allothreonine and 63% DL-threonine is reached, at which point the solubility temperature has a minimum value. Mixtures containing increasing amounts of DL-threonine have higher solubility temperatures until finally the solubility temperature of DL-threonine is reached. With mixtures having compositions represented by the left-hand portion of the curve, the last material to dissolve as the temperature is raised is the DL-allothreonine. At the minimum point on the curve, the DL-threonine and the DL-allothreonine are present in the ratio of their solubilities, and both dissolve together. On the right-hand portion of the curve, the DL-threonine is the last component to dissolve. Over part of the diagram an observed solubility temperature can represent two compositions. These can be differentiated by determining the change in solubility temperature when a mixture of one of the pure components with the unknown sample is run.

In the case of the DL-threonine–DL-allothreonine mixture, each side of the solubility temperature–composition curve can be closely approximated by plotting the solubility temperature of each pure component determined separately with amounts of the component equal to that amount which would be present if the mixture of isomers was used. This is represented by the dashed line of Figure 3, and demonstrates how each of these particular isomers dissolves almost independently of the other.

"Binary" mixtures containing small amounts of other components can be analyzed successfully, providing the other components are sufficiently soluble to dissolve before the end point is reached. This is done by analyzing for one component by determining the solubility temperature in the usual way, then

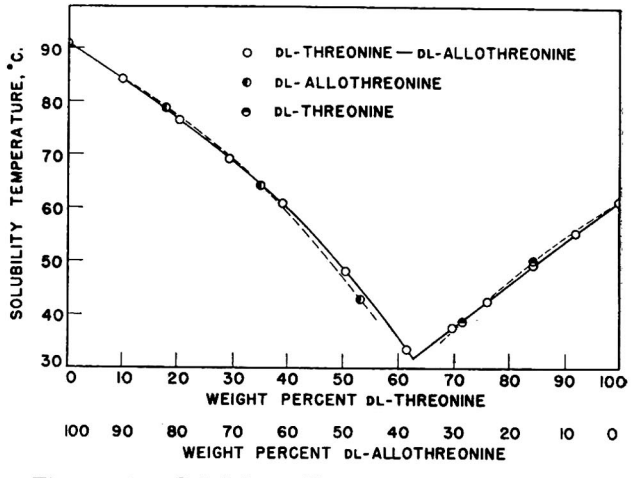

Figure 3. Solubility Temperature–Composition Diagram for DL-Threonine–DL-Allothreonine System

Solid line. DL-Threonine–DL-allothreonine mixture with water to amino acid ratio of 3:1

Dashed line. DL-Allothreonine alone (*left*) and DL-threonine alone (*right*) with % of amino acid used relative to amount required for 3:1 ratio plotted as abscissa

analyzing for the second component by determining the solubility temperature of a mixture of the unknown and the second component. This mixture must contain sufficient of the second component so that the solubility temperature is determined on the part of the curve where the second component is the last material to dissolve. The percentage of the second component in the mixture can be calculated from the following equation:

$$P_2 = \frac{P_m(W + A) - 100A}{W}$$

where P_2 equals the per cent of the second component in the original sample, P_m equals the per cent of the second component observed in mixture of sample and second component, W equals weight of original sample, and A equals weight of added second component.

If the percentage of the first and second components is known, the difference between the sum of these and 100 represents the percentage of the other components present, providing these other components are not present in sufficient amounts to alter seriously the solubilities of the first two components.

In the analysis of mixtures consisting predominantly of two previously characterized components, this method has decided advantages over the procedure of Northrop and Kunitz, which fails to distinguish more than one component when two components are present in the same ratio as their solubilities. Thus the Northrop and Kunitz (*7*) procedure would show a mixture of 37% allothreonine and 63% threonine to be a homogeneous single material, especially because the relative solubilities of these isomers are independent of temperature and only aqueous solvents can be used. If it is not possible to obtain the pure components to construct the solubility temperature–composition diagram, then the solubility temperature procedure obviously fails, whereas the procedure of Northrop and Kunitz will often be successful.

DETERMINATION OF SOLUBILITY

From solubility temperatures determined with different ratios of solvent, approximate solubility data can be calculated. Data obtained by this dynamic method will approximate data obtained under equilibrium conditions, but they will not be identical. In the case of DL-threonine, the data closely fit the equation $\log S = 2.1381 + 0.005858t + 0.000007t^2$ over the temperature range 14° to 61° C. where S is the solubility expressed in grams per 1000 grams of water and t is the temperature in degrees centigrade. For DL-allothreonine, the equation is $\log S = 1.895 + 0.00688t$ over the temperature range 43° to 91° C. These data are believed to be accurate to within at least 1%.

EXPERIMENTAL

Purification of Amino Acids. The amino acids were recrystallized as recommended by Dunn and Rockland (*6*).

The DL-alanine (Bios Laboratories, Inc.) was recrystallized six times. Its solubility temperature at 6 to 1 was: two recrystallizations, 25.2° C.; four, 26.1° C.; six, 26.3° C. DL-Aspartic acid (Eastman Kodak Company) was recrystallized four times. Its solubility temperature at 25 to 1 was: two recrystallizations, 70.9° C.; four, 70.8° C. L-Histidine hydrochloride monohydrate (Pfanstiehl Chemical Company) was recrystallized twice. Analysis calculated for $C_6H_{12}O_3N_3Cl$: C, 34.37; H, 5.77. Found: C, 34.39; H, 5.80. Solubility temperature at 6 to 1: unrecrystallized, 23.2° C.; recrystallized twice, 23.5° C.

DL-Leucine (Mann Fine Chemicals, Inc.) after two recrystallizations had a solubility temperature at 25 to 1 of approximately 105.5° C. as determined by immersing the sealed sample tube, fastened with rubber bands to the ends of an ordinary 110° C. thermometer, in an oil bath and shaking by hand. DL-Phenylalanine (Mann Fine Chemicals, Inc.) was recrystallized six times. Its solubility temperature at 25 to 1 was: two recrystallizations, 80.4° C.; six, 81.5° C.

DL-Threonine (Eastman Kodak Company, Merck, and Interchemical Company) was recrystallized ten times by dissolving it in the smallest amount of boiling water, then adding three volumes of absolute ethyl alcohol and allowing it to cool to room temperature and stand 24 hours. The neutral equivalent by formol titration was 119.4; calculated, 119.1. The solubility temperature was unchanged by the last four recrystallizations. DL-Allothreonine was prepared by the procedure of Adkins and Reeve (*1*) and recrystallized nine times. The neutral equivalent by formol titration was 119.3; calculated, 119.1. The solubility temperature was unchanged by the last two recrystallizations. DL-Valine (Mann Fine Chemicals, Inc.) was recrystallized four times. Its solubility temperature at 6 to 1 was: two recrystallizations 94.1° C.; four, 94.5° C.

Table II. Solubility Temperature Data

(3 to 1 ratio of water to total solids)

DL-Allothreonine, %	DL-Threonine, %	Solubility Temperature, ° C
Pure	...	91.2
80.7 + 19.3 sucrose	...	78.2
80.8 + 19.2 glycine	...	76.3
95.0 + 5.0 NH_4Cl	...	86.8
79.9 + 20.1 NH_4Cl	...	73.1
90.1	9.9	84.3
79.7	20.3	76.8
70.6	29.4	69.2
60.9	39.1	61.0
49.6	50.4	48.6
38.4	61.6	33.8
30.4	69.6	37.9
23.4	76.6	43.3
15.8	84.2	49.7
7.9	92.1	55.4
...	100	61.1
	Ratio of Water to Amino Acid	
Pure DL-threonine	6.00	14.0
	4.20	39.0
	3.56	50.0
	3.00	61.1
Pure DL-allothreonine	6.37	43.1
	4.61	64.5
	3.66	78.9
	3.00	91.2

Solubility temperature data for Figures 2 and 3 are given in Table II.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the financial assistance of the Biological Laboratories, Organic Chemical Department, E. I. du Pont de Nemours & Company which made this research possible. Many of the solubility temperatures were either checked or determined by Charles Haber. The microanalyses were performed by Mary Aldridge.

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RECEIVED December 13, 1949.

Correction

In the article on "Iodometric Determination of Resorcinol" [ANAL. CHEM., **22**, 585 (1950)] in the first column, last paragraph, the next to the last sentence should read: "The mixture was stirred until solution was complete and then was diluted to 0.5 liter." In the second column, first equation, $3I_2$ should have been used instead of $3I_3$.

H. H. WILLARD

Quantitative Determination of Amino Acids by Iodometric Titration of Their Copper Salts

Reinvestigation of the Method of Pope and Stevens

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A reinvestigation of the method of Pope and Stevens for the determination of amino nitrogen in protein hydrolyzates by means of the iodometric determination of the soluble copper salts of the amino acids has led to a better definition of some of the variables that made difficult the duplication of their results. By the use of washed copper phosphate, fourteen of nineteen naturally occurring amino acids were determined with an accuracy of about ±1% in quantities of 0.05 to 0.20 millimole, three gave high but consistent results (102%), and histidine and cystine could not be determined satisfactorily. The dead-stop end-point method was used to decrease subjective error of iodometric determination.

THE quantitative determination of amino acids after their separation from protein hydrolyzates by chromatographic or other methods has usually been made by colorimetry, titration, determination of amino nitrogen by Van Slyke's methods, etc., but little attention has been given to their iodometric determination by means of the copper salts. However, the work of Kober and Sugiura (*5*), Kober (*4*), and Pope and Stevens (*10*) indicates that in most cases two molecules of an amino acid react stoichiometrically with one cupric ion to form a blue-colored copper salt. Because the copper salt is usually soluble, the quantity of amino acid can be determined by reacting a solution of amino acid with a suspension containing an excess of precipitated copper hydroxide or copper phosphate, removing the excess copper reagent by filtration or centrifugation, and then determining the soluble copper iodometrically in the supernatant liquid.

In order to assess the usefulness of the method, Pope and Stevens' procedure with copper phosphate was tried on several pure amino acids, but satisfactorily reproducible results were not obtained. The difficulty was traced to the presence of excess phosphate ion in the copper phosphate suspension which had been prepared in the manner of Pope and Stevens by adding a solution containing cupric ion to one containing excess phosphate ion; by halving or doubling the initial amount of phosphate it was possible to obtain results which ranged from 98 to 107%, respectively, of the expected values. However, copper phosphate which had been washed free of excess phosphate and suspended in a borax buffer gave satisfactory and reproducible determinations. Martin and Mittelman (*7*), who did not attempt to obtain highly accurate results, used a copper phosphate suspension in which the concentration of excess phosphate ion was controlled.

Woiwod (*15*), in a recent application of the method to the determination of amino acids from paper chromatograms, also regulated the concentration of excess phosphate ion and prepared the copper phosphate somewhat differently than Pope and Stevens. He, too, noted that the concentration of phosphate influenced the results.

Because the use of washed copper phosphate appeared to offer advantages over the method of Pope and Stevens, the investigation was extended to include nineteen naturally occurring amino acids, eight of which had not been studied by Pope and Stevens. The quantities of amino acid which were taken for the present work were about one half to one tenth of those used by Pope and Stevens, about the same as those used by Albanese and Irby (*1, 2*), but much greater than the submicro amounts used by Martin and Mittelman (*7*) and Woiwod (*15*). The precision of the procedure has been further improved by the use of the dead-stop end-point method of Foulk and Bawden (*3*) in the iodometric determination of the copper.

REAGENTS AND SOLUTIONS

Sodium Thiosulfate, 0.005 *N*. Solutions were prepared by dissolving 1.3 grams of sodium thiosulfate pentahydrate and 1.8 grams of sodium borate decahydrate, reagent grade, in 1 liter of distilled water.

Copper Metal. Electrolytic foil (0.002 inch) and 20-gage uncovered wire. Surface corrosion was carefully removed before use.

Potassium Iodide, C.P.

Acetic Acid Solution. Sixty milliliters of glacial acetic acid were diluted with 900 ml. of distilled water.

Cupric Chloride Solution. A 2.8-gram sample of cupric chloride dihydrate, C.P., was dissolved in 100 ml. of distilled water.

Sodium Phosphate Solution. A 13.7-gram sample of tribasic sodium phosphate dodecahydrate, reagent grade, was dissolved in 200 ml. of distilled water and the solution was filtered. In the course of several weeks the solution became turbid. Although the turbidity may be without deleterious effect, the solution was discarded when it became noticeably turbid.

Borax Buffer, pH 9.1. This buffer was prepared by dissolving 76.4 grams of powdered sodium borate decahydrate in 4 liters of water.

Sodium Chloride, C.P.

Washed Copper Phosphate. To 40 ml. of sodium phosphate solution, 20 ml. of cupric chloride solution were added with swirling. The resulting suspension of copper phosphate was centrifuged for 5 minutes, the supernatant solution was removed and replaced with an equal volume of borax buffer, and the copper phosphate was resuspended. The suspension was again centrifuged and the washing was repeated. Finally, the copper phosphate was suspended in 100 ml. of borax buffer and 6 grams of solid sodium chloride were added.

Amino Acid Solutions. Solutions (usually 0.02 to 0.03 molar) were made up in distilled water in quantities of 25 to 100 ml.

Sources of Amino Acids. The sources of the amino acids were as follows: L-arginine free base, L-histidine free base, hydroxy-L-proline, L-phenylalanine, and DL-serine from Mann Fine Chemicals, Inc.; L-cystine and L-tryptophan from Van Camp Laboratories, Terminal Island, Calif.; DL-isoleucine, DL-threonine, and DL-valine from Winthrop Chemical Company, Inc.; DL-methionine from U. S. Industrial Chemicals, Inc.; DL-alanine and L-tyrosine from Amino Acid Manufactures (University of California at Los Angeles); L-proline from General Biochemicals, Inc.; glycine from B. L. Lemke Company (recrystallized); L-glutamic acid from International Minerals and Chemi-

cals Corporation (recrystallized); L-aspartic acid from hydrolysis of asparagine (recrystallized); and L-leucine from B. L. Lemke Company (purified by the method of Thomas and Niemann, *13*). All samples were dried to constant weight before use. Ammonia and halogen determinations by the procedure of Stoddard and Dunn (*12*) showed less than 0.04% of either in all samples and less than 0.004% in most. The purity of one sample of hydroxyproline was suspected because it gave higher recoveries than a second sample; indeed, starch chromatography (*8*) showed the presence of glycine (or possibly serine) in sufficient amount to account for the higher recovery. Data from this sample have been omitted.

RECOMMENDED PROCEDURE

On the basis of the results which were obtained from the variations discussed below, the following recommended procedure was devised.

Standardization of Sodium Thiosulfate. The method of Kolthoff and Sandell (*6*) for the standardization of 0.1 *N* sodium thiosulfate was modified as follows: The copper solution as there prepared was diluted eightfold and a 5-ml. aliquot portion (copper concentration approximately 0.6 mg. per ml.) was added to a solution of 5 grams of potassium iodide in 32 ml. of acetic acid solution. The resulting iodine was titrated with approximately 0.005 *N* thiosulfate using the dead-stop end-point method, discussed below. The volume used in a titration was 8 to 10 ml. When duplicate determinations with a single standard solution were made, the titrations rarely differed by more than 0.005 ml.; when two or three standard solutions were used the spread was only 0.1 to 0.2%. The two samples of copper yielded identical standards. Restandardization of a solution of thiosulfate after 26 days showed a decrease in normality of about 0.1%.

Determination of Amino Acids. The following procedure is applicable to an amino acid which forms a soluble copper salt.

A 5-ml. portion of washed copper phosphate suspension and a 5-ml. portion of a solution of the amino acid whose concentration was to be determined were pipetted successively into a 15-ml. centrifuge tube. The solution was allowed to stand for 5 minutes with occasional stirring, the centrifuge tube was capped, and the excess copper phosphate was removed by centrifuging for 5 minutes in an International clinical centrifuge (Model 414). A 7-ml. portion of the supernatant liquid was pipetted into a solution of 5 grams of potassium iodide in 32 ml. of acetic acid solution, which had been titrated free of traces of iodine, and the resulting iodine was titrated with standard sodium thiosulfate.

When the amino acid forms an insoluble or partially soluble copper salt (as do leucine, methionine, phenylalanine, and tryptophan), the procedure must be modified in order to obtain a soluble copper salt, by adding to the amino acid solution a known amount of glycine in the molar ratio indicated in Table I. The presence of glycine produces a soluble salt, apparently through the formation of a double salt. The method described above for soluble copper salts is applied to a portion of the mixture. The quantity of copper in solution due to the known amount of glycine is then subtracted from the total copper in solution to yield that due to the normally insoluble amino acid and hence the amount of that amino acid can be calculated.

Cystine cannot be determined in this way, because it forms a very insoluble salt even in the presence of glycine.

Determination of the Blank. During the determination of an amino acid a stable end point was obtained, but during the determination of the blank the end point drifted unless copper had previously been added. The drifting end point apparently was caused by air oxidation of iodide, inasmuch as it could be reduced by passing nitrogen over or through the solution. Neither the current across the electrodes nor the stirring of the solution was responsible. Therefore, the blank determination was run in the following manner in order to obtain consistent results.

Approximately 1 ml. of copper solution (concentration about 0.5 mg. per ml.) was added to the potassium iodide in acetic acid solution, and after the iodine had been titrated a sample of the supernatant liquid from the blank was added and the iodine was again titrated. The blanks generally were in the range of 0.02 to 0.05 ml. of 0.005 *N* thiosulfate—that is, 0.5% or less of the usual volume of titration.

DEAD-STOP END-POINT METHOD

When 0.005 *N* solutions of sodium thiosulfate are used to titrate iodine with starch as the indicator, the choice of the end point is somewhat uncertain because the color fades only gradually as successive drops are added. Therefore, in order to decrease the subjective error and to increase the precision of the titrations, the starch end point was rejected in favor of the dead-stop end-point method of Foulk and Bawden (*3*). The apparatus for this end point as described by Wernimont and Hopkinson (*14*) was essentially duplicated in this work. A sensitivity of approximately 20 scale divisions per 0.02 ml. of 0.005 *N* thiosulfate was obtained by adjusting the potential drop across the electrodes to about 15 millivolts.

The very dilute thiosulfate solution caused some difficulty in the use of the dead-stop end point: It was observed that as thiosulfate was added the end point was approached but never quite reached. As the end point was neared and the indicator light of the galvanometer began to move onto the scale, the sensitivity was about 20 scale divisions per drop. The sensitivity decreased with successive drops until, within a few scale divisions of the dead-stop end point, several drops were required to move the indicator light one scale division and the dead-stop point itself usually could not be attained. Pring and Spencer (*11*), Pagel and Miers (*9*), and Wernimont and Hopkinson (*14*), who used solutions as dilute or even more dilute, make no mention of such a difficulty.

To overcome this effect the potassium iodide was first dissolved in the acetic acid solution. Then, before the addition of the copper, the traces of iodine (about 0.001%) which are present in potassium iodide in sufficient quantity to move the indicator light off the scale were titrated with thiosulfate to an arbitrary zero in a range in which the galvanometer was still sensitive. The copper solution was then added and the titration was carried to the same point.

The quantity of glacial acetic acid in the solution (2 ml.) was chosen after variations from 0.5 to 5 ml. were found to give identical results.

The quantity of potassium iodide is somewhat more critical. If only 1 gram of potassium iodide per milligram of copper is used precipitation of cuprous iodide usually occurs, with resultant decrease in sensitivity of the dead-stop end point. It is essential that the cuprous iodide be kept in solution by using 1.5 to 2 grams of potassium iodide per milligram of copper. Greater ratios of iodide to copper tend to give a slightly high titer. Pring and Spencer (*11*) report satisfactory results when the ratio by weight of potassium iodide to copper is only 5 to 1.

RESULTS

The results given in Table I were obtained when the recommended procedure was applied to solutions of pure amino acids. For twelve of the nineteen amino acids which were studied the average amount found was within 99 to 101% of that taken, and for lysine and proline the values were only slightly outside this range. Leucine, threonine, and hydroxyproline gave consistently high results (102%), regardless of whether washed copper phosphate or copper hydroxide was used. Histidine behaved erratically and does not seem to be amenable to determination by this method. Cystine forms a salt so insoluble that the addition of glycine in reasonable proportion is of no help.

DISCUSSION OF PROCEDURE AND EFFECT OF VARIATIONS

Variations in Preparation of Copper Phosphate. The initial runs by the method of Pope and Stevens (*10*) followed their procedure except in two details: A borax buffer without the addition of hydrochloric acid was used and the excess copper phosphate was removed by centrifugation rather than by filtration. When the borax buffer (pH 9.1) and the buffer of Pope and Stevens (pH 8.65) were tested with glycine no difference was detected. The borax buffer had the advantage of simplicity of preparation.

Complete removal of excess copper phosphate by centrifugation was attained only when sodium chloride was added to the suspension, and this added sodium chloride was not without effect on the results. When unwashed copper phosphate was used and was removed by filtration without the addition of sodium chloride (Pope and Stevens' method), the result of 110% was not at all in accord with the 101% found by Pope and Stevens. Substitution of washed copper phosphate yielded a more reasonable though still high value of 103.5%, but when sodium chloride was added to the washed copper phosphate essentially theoretical results were obtained, and filtration and centrifugation were equally effective (98.8 and 99.2%, respectively). Centrifugation was chosen because of its greater simplicity and rapidity. Whereas a concentration of 1.5% of sodium chloride in the final reaction mixture is insufficient to produce complete removal, as much as 6% seems to be without adverse effect: A concentration of 3% was chosen for the recommended procedure.

The copper phosphate suspension was found to be stable for at least 2 weeks, although there was a slight rise in the values over this period. Thus, when freshly prepared material was used, the values with glycine were 98.8 and 98.5%; after 3 days, 99.5 and 99.4%; after 7 days, 100.3 and 100.2%; and after 14 days, 100.2%.

Determinations with Copper Hydroxide. Pope and Stevens introduced the use of copper phosphate because of their inability to obtain satisfactory results with copper hydroxide. However, when washed copper phosphate was found to give high results with some amino acids, a considerable number of determinations were made in the present work with the hydroxide as the insoluble copper compound in the hope that more accurate results would be obtained.

Copper hydroxide was prepared essentially by the method of Kober (*4*). The mixture of 5 ml. of a 5% solution of copper chloride, 5 ml. of a 1% solution of sodium chloride, and 40 ml. of water was treated with slightly less than an equivalent amount of 0.5 *N* sodium hydroxide. The copper hydroxide was centrifuged and suspended in 60 ml. of borax buffer. The recommended procedure was then followed from this point.

Comparison of the results from copper hydroxide and washed copper phosphate showed fair agreement, but copper hydroxide

Table I. Determination of Amino Acids by Means of Copper Salts Using Recommended Procedure

The determinations of each amino acid in this table were made in the order given over a period of about 2 months. Bracketed results were determined on a single solution over the course of a week or 10 days. Values in parenthesis have been omitted from the average because of wide deviation.

Amino Acid	Alanine	Arginine Free Base	Aspartic Acid	Glutamic Acid			Glycine			Hydroxyproline	Isoleucine	Leucine[a]
Approx. sample size, mg.	12	24	17	9	20	30	4	10	16	18	17	6
Individual determinations, % found	99.9 100.2 100.3 100.3 100.3 100.4 100.1 100.1 99.7 99.9 99.8 99.7 100.1 100.1	99.6 99.6 99.2 99.2 99.4 99.4 99.4 99.4 99.3 99.3 98.0[d] 97.7[d] 97.4[e] 97.8[e]	101.6[c] 101.6[c] 101.3 101.3 100.9 100.8 100.9 100.8 101.0 100.7 100.2 99.9 100.5 100.8 100.4 100.6	100.2 100.2 100.6 100.4 100.4 100.3 100.1 100.1 99.3	100.0 100.4 100.4 100.6 101.1 100.3 100.2 101.0 101.0 100.2	99.8 100.1 100.3 100.1 (97.5) (98.5) 100.1 100.2 100.2	100.2 99.5 99.8 99.8 98.6 98.4 97.5 97.7 98.6 99.0 98.8 99.3 98.8 98.8 100.0 99.0 99.0	99.4 99.7 99.5 98.8 98.5 99.5 99.4 98.8 99.1 99.2 99.1 99.6 99.4 99.4 99.4 99.9 99.6	99.9 100.2 100.4 100.2 (97.1) 98.5 99.6 99.3 99.5 (96.4) 99.0 99.3 99.3 99.9 99.7 99.6 99.5 99.3	102.0 102.1 101.9 101.9	101.2 101.2 100.5 100.8 100.8 100.2 100.1 101.0 101.2 100.4 100.2	101.8 102.2 101.3 102.3 101.9 102.6 101.9 103.0 103.0
Av. % found	100.1	99.4	100.8	100.2	100.5	100.1	99.0	99.3	99.6	102.0	100.7	102.2
Av. deviation	±0.2	±0.1	±0.3	±0.1	±0.3	±0.1	±0.6	±0.3	±0.4	±0.1	±0.4	±0.4

	Lysine Hydrochloride			Methionine[b,f]	Phenylalanine[b,g]	Proline	Serine	Threonine	Tryptophan[b,h]	Tyrosine[i]	Valine
Approx. sample size, mg.	11	23	32	7	10	15–20	15	16	13	25	15
Individual determinations, % found	98.5 99.1 98.9 98.2	99.5 99.8 98.0 98.0 98.6 97.8 99.3 98.9 98.9 99.5 99.2 98.7	(101.8) 98.9 99.5 99.3 98.9	(103.8) (103.0) (102.9) 100.9 100.9 100.8 100.5 100.9 100.3 100.1 100.3 101.1	100.9 99.9 98.8 100.8 100.9 99.9 99.9 99.5 99.5 100.8 101.2 100.0	99.3 99.3 99.5 98.5 98.6 98.7 98.9 98.3 98.5 98.7 98.6 98.9 98.9 98.9 98.7	100.9 100.7 100.5 100.5 100.0 100.1 99.9 100.0 100.1 100.3 100.3 100.1 99.7	102.8 103.1 102.0 102.0 101.9 102.1	(96.8) (91.7) 100.2 101.1 99.8 99.9 100.0 99.3 99.9 98.9 99.0 98.7 99.1	99.8 100.3 100.6 100.6 100.8 100.5 101.4 100.9 100.9 101.0 100.7 100.2	100.9 100.8 101.1 101.3 101.2 (103.0) 101.0 101.0 100.8 100.7 100.7 100.8
Av. % found	98.7	98.9	99.2	100.6	100.2	98.8	100.2	102.3	99.6	100.6	100.9
Av. deviation	±0.3	±0.5	±0.3	±0.3	±0.6	±0.3	±0.3	±0.4	±0.5	±0.3	±0.2

[a] 2 to 1 glycine-leucine solution. Ratio is in moles here and in *f*, *g*, and *h*.
[b] Assumed glycine recovery was 99.3%.
[c] Aspartic acid solution neutralized with sodium hydroxide to faint blue color of thymolphthalein as internal indicator.
[d] Not included in average. Determination made on sample of L-arginine monohydrochloride, Lemke.
[e] Not included in average. Determination made on sample of L-arginine monohydrochloride, Van Camp Laboratories.
[f] 2 to 1 glycine-methionine solution.
[g] 3 to 2 glycine-phenylalanine solution.
[h] 1 to 1 glycine-tryptophan solution.
[i] 0.5 *N* sodium hydroxide added to faint blue color of thymolphthalein internal indicator in order to dissolve tyrosine.

tended to give high values, especially with the acidic amino acids. In addition, it is inferior to copper phosphate because the reproducibility of both determinations and blanks is poorer, the blanks are higher, the stability is limited to about 8 hours, and centrifugation of the excess tends to be incomplete even with added sodium chloride.

Table II. Effect of Acid or Base in Solution on Determination of Glycine

Five milliliters of glycine solution (10 mg. per ml.) were diluted to 25 ml. after addition of acid or base and 5 ml. of the resulting solution were used. Each blank was made up in same way as run. Glycine in aqueous solution at this concentration gives average value of 99.3 ± 0.3%

Ratio of HCl to Glycine, Equiv.	% Glycine Found	Ratio of NaOH to Glycine, Equiv.	% Glycine Found
2	98.0	2	100.0
5	38.3	5	89.6
10	All $Cu_3(PO_4)_2$ dissolved	10	18.1
10[a]	99.9	10[a]	99.3

[a] Neutralized to faint blue color of thymolphthalein internal indicator.

Time of Reaction. The length of time between the mixing of the reagents and the centrifugation of the excess copper phosphate was varied from 1 to 30 minutes with glycine as the test substance and these results were obtained: 1 minute, 99.4 and 99.6%; 5 minutes, 99.2 and 99.2%; 30 minutes, 98.9 and 98.9%. Although there is a slight decrease, all results fall within the spread of values which were obtained from a large number of determinations with the recommended time of 5 minutes (Table I). To the eye, at least, the reaction appears to be instantaneous; there is no evident increase in color intensity over that which occurs immediately on mixing the reagents.

Variation in Amount of Amino Acid. In most of the runs the amount of copper phosphate was about double that required to form the copper salt. In order to determine the effect of varying the amount of the amino acid when the quantity of copper phosphate is kept constant, experiments in which the quantity of amino acid was approximately halved or increased by half were made with glycine, glutamic acid, and lysine hydrochloride, as representative amino acids. By using these amounts the titration volume was not decreased to the point where inordinate errors occurred nor was the copper phosphate exhausted. The results are shown in Table I. In the case of glycine and lysine the percentage found increases slightly with increase in the amount of amino acid, whereas glutamic acid gives random results. However, when the spread of values at the different concentrations is considered the trend of the averages seems to be too slight to be significant, and it may be concluded that satisfactory results may be obtained when the amount of amino acid is approximately 0.05 to 0.20 millimole, the quantity of copper phosphate is about 20 mg., and the volume of reaction mixture is 10 ml.

Effect of Acid or Base in Amino Acid Solution. Although aqueous solutions containing amino acids only were used to obtain the data in Table I, it is unlikely that pure aqueous solutions of this sort would be obtained in most cases as the final step in an isolative procedure. Rather acid or base might often be present. Consequently, the effect of acid or base in the solution was studied, with the results given in Table II. Apparently acid or base in quantity greater than twice the equivalent amount of amino acid renders the procedure useless and acid is more detrimental than base. When neutralized, the original presence of as much as 10 times the equivalent amount of acid or base is without effect; the results are within the range obtained with pure aqueous solutions of the amino acid.

Histidine. Kober and Sugiura (*5*) suggested and Pope and Stevens (*10*) also came to the conclusion that histidine forms a salt in the ratio of 2 cupric ions to 3 molecules of histidine rather than in the normal ratio of 1 cupric ion to 2 molecules of amino acid. That the copper histidine salt is different is perhaps substantiated by the fact that its color is much greener than that of the others, which in general at the same concentration show differences only in the intensity of the blue color. If a solution of the histidine salt is allowed to stand overnight its color becomes still more green and a greenish brown precipitate forms. Although the above authors obtained good results with histidine, the present authors were unable to do so. When the recommended procedure was used the quantity of histidine found was about 92% of that taken if the 2 to 3 ratio is used as the basis of calculation. The results were very sensitive to the pH of the buffer; they increased from about 92% to about 107% when the pH was changed from 9.1 to 10.1. It would seem that quantitative results might be obtained by control of the pH. However, the slope of the curve of percentage against pH is so steep that it seemed improbable that proper control of pH could be achieved, and so experiments were discontinued.

Amino Acids Forming Insoluble Copper Salts. As already noted, cystine, leucine, methionine, phenylalanine, and tryptophan form insoluble or only partially soluble salts with copper, and accordingly the device of Pope and Stevens was used in order to form a soluble salt: A known amount of glycine was added to the amino acid to be determined. That this soluble salt (presumably the double salt) is unstable is shown by the formation of crystals (probably of the less soluble copper amino acid) on standing. However, there is adequate time for the titration before precipitation begins. Although Pope and Stevens added glycine in an amount equivalent to four times the other amino acid, smaller ratios could be used at the concentrations which were studied, as shown in Table I. The error caused by the addition of glycine was thus reduced. The copper salt of cystine is so insoluble that a precipitate formed immediately when 2 to 1 glycine-cystine solution was added to copper phosphate suspension, and hence the study of the determination of cystine by means of this procedure was abandoned.

Valine and isoleucine tend to precipitate the copper salt on standing or when the concentration of amino acid is increased, but at the concentrations which were used the addition of glycine was unnecessary.

When alanine was substituted for glycine in these experiments it was found to be inferior in its action.

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THE HYDROXYLAMINE NUMBER

Application to the Identification of Ketones

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The "hydroxylamine number" is defined as "the number of milligrams of potassium hydroxide which is equivalent to the hydroxylamine required to oximate the carbonyl function in 1 gram of sample." The present study, which is limited to pure samples of ketones of various structural types for which the hydroxylamine number is an invariant quantity, has demonstrated that the concept can be readily applied to problems in the qualitative and quantitative identification of ketones. It presents an effective teaching aid in courses in qualitative organic analysis. It is applicable to simple and complex ketones. Internal interferences due to steric factors, cyclization with formation of isoazoxalones, conjugated unsaturation, and hydrogen bonding have been studied and are discussed.

IN COURSES of qualitative organic analysis, aldehydes and ketones are most frequently identified by the derivatives they form with hydrazine or one of its substitution products (*12*). It is, indeed, surprising in view of the many excellent, rapid, and accurate quantitative analyses of organic compounds via functional groups which are available (*13*) that, pedagogically, practically the only methods applied in qualitative organic analysis are the time-worn, but very important, neutralization and saponification equivalents. To some extent we find even these simple quantitative tools neglected in emphasis upon the "solid derivative" for identification purposes. One of the authors (E. L.), in his classrooms, has striven to inculcate on the student the more quantitative aspects involved in the identification of organic compounds. The present paper is a summary of the application of the hydroxylamine titration of the carbonyl function to the identification of ketones and introduces the concept of the "hydroxylamine number" for such purposes. The present study is limited to pure compounds.

The hydroxylamine number, in analogy to such terms as neutralization number, saponification number, iodine number, etc., is defined as "the number of milligrams of potassium hydroxide which is equivalent to the hydroxylamine required to oximate the carbonyl function in 1 gram of sample." Because the functionality of a pure organic compound is an invariant quantity, the hydroxylamine number becomes as important a constant for the identification of a carbonyl compound as the preparation of an independent derivative. It thus can serve as an important tool in problems of the qualitative and quantitative identification of aldehydes and ketones. It is less time-consuming and more sparing of material, because micro and semimicroprocedures can be used. The determination of the hydroxylamine number of a carbonyl compound will enable an analyst to compute the equivalent weight of the compound. If the compound has been characterized previously, he may check its identity as determined by the classical method or, if the compound is new, may compare the equivalent weight to the molecular weight as measured by the simple and rapid Rast procedure (*12*).

The requirements for the identification of a pure organic compound must also be based on physical constants, spectra, or other "fingerprint" data peculiar to a particular compound. One usually has such knowledge prior to the preparation of a solid derivative or the determination of its hydroxylamine number, and thus isomeric carbonyl compounds—e.g., valeraldehyde, diethyl ketone, methyl ethyl ketone, etc.—can be differentiated and identified. With such *a priori* knowledge it is often possible to identify carbonyl compounds in mixture without isolation.

HISTORICAL

The concept of a hydroxylamine number is not new. This was expressed as a "carbonyl value" by Stillman and Reed (*15*) in 1932 and applied by them to the determination of aldehydes and ketones in essential oils. Published in an obscure journal, the concept introduced by Stillman and Reed has found no application such as pointed out in the present paper and has never been used for instructional purposes in courses of qualitative organic analysis. This early paper (*15*) contains an excellent and detailed review of the hydroxylamine titration as known up to that time. More recent studies on volumetric oximation procedures have been published by Sabetay (*10*), Martin and co-workers (*5*), Siggia (*13*), and Mitchell and co-workers (*6*), the latter employing the Karl Fischer reagent in conjunction with hydroxylamine. Oppenauer (*8*) has applied the hydroxylamine titration in following the reaction velocity of the dehydrogenation of cholesterol to cholestenone, and Light (*4*) for the determination of carbonyl compounds in bile preparations. Byrne (*2*) has recently developed an interesting procedure for the determination of microquantities of acetone based on the change in pH caused by the liberation of hydrochloric acid by reaction with hydroxylamine hydrochloride, the method depending upon the calibration of a standard curve.

Table I. Stability of Hydroxylamine Reagent

Age of Reagent, Days	0.05 *N* HCl Required, Ml.[a]
0	30.75
3	28.75
5	27.15
10	26.25
15	18.83
20	15.75

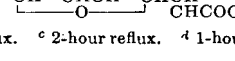
[a] For 50 ml. of reagent.

Table II. Dialkyl Ketones, Cyclic Ketones, and Polyketones

Compound	Structure	Hydroxylamine Number Calcd.	Found	% deviation
Acetone	CH_3COCH_3	966	965	0.14
Diethyl ketone	$C_2H_5COC_2H_5$	651	645	0.92
Di-*n*-butyl ketone	$C_4H_9COC_4H_9$	394	388	1.52
Methyl isobutyl ketone	$CH_3COCH_2CH(CH_3)_2$	561	559	0.36
Pinacolone	$CH_3COC(CH_3)_3$	561	558	0.54
9-Heptadecanone	$C_8H_{17}COC_8H_{17}$	220	219	0.45
Cyclohexanone	$CH_2(CH_2)_4CO$	572	579	1.2
m-Methylcyclohexanone	$CH_2(CH_2)_2CH(CH_3)CH_2CO$	500	497	0.6
Acetylacetone	$CH_3COCH_2COCH_3$	1120[a]	1132	1.07
Acetylcaproyl	$CH_3(CH_2)_4COCOCH_3$	789[a]	791	0.25
Benzoylacetone	$C_6H_5COCH_2COCH_3$	692[a]	410[b]	..
12,13-Tetracosadione	$C_{11}H_{23}COCOC_{11}H_{23}$	307[a]	309	0.65

[a] Calculated for two carbonyl groups.
[b] Stable oximation value.

Table III. Keto Esters, Keto Carboxylic Acids, Hydroxyketones, and Unsaturated Ketones

Compound	Structure	Hydroxylamine Number Calcd.	Found	% deviation
Ethyl acetoacetate	$CH_3COCH_2CO_2C_2H_5$	431	435	0.93
Ethyl *n*-propylacetoacetate	$CH_3COCH(C_3H_7)CO_2C_2H_5$	325	327	0.62
Ethyl α-lauroyl laurate	$C_{11}H_{23}COCH(C_{10}H_{21})CO_2C_2H_5$	142	145	2.06
Ethyl acetonedicarboxylate	$CO(CH_2CO_2C_2H_5)_2$	277	274	1.09
Ethyl benzoylacetate	$C_6H_5COCH_2CO_2C_2H_5$	292	291.5	0.17
Ethyl benzoylformate	$C_6H_5COCO_2C_2H_5$	315	310.5	1.43
Benzoin acetate	$C_6H_5CH(O_2CCH_3)COC_6H_5$	221	219[a]	0.91
Ethyl levulinate	$CH_3CO(CH_2)_2CO_2C_2H_5$	389	390	0.26
β-Benzoyl propionic acid	$C_6H_5CO(CH_2)_2COOH$	315	318	0.95
Diacetone alcohol	$CH_3COCH_2C(OH)(CH_3)_2$	483	477	1.24
Benzoyl carbinol	$C_6H_5COCH_2OH$	412	408	0.98
Benzoin	$C_6H_5COCH(OH)C_6H_5$	264	260.5	1.33
o-Hydroxyacetophenone	o-$(HO)C_6H_4COCH_3$	412	409	0.73
p-Hydroxypropiophenone	p-$(HO)C_6H_4COC_2H_5$	374	377	0.80
2-Aceto-1-naphthol	$C_{10}H_6(1)(OH)(2)(CH_3CO)$	301	301[b]	0.00
Benzalacetophenone	$C_6H_5CH{=}CHCOC_6H_5$	270	270	0.00
Cinnamalacetophenone	$C_6H_5(CH{=}CH)_2COC_6H_5$	239	242.5[c]	1.46
Dibenzalacetone	$[C_6H_5CH{=}CH]_2CO$	239	238.5[d]	0.21
Dicinnamalacetone	$[C_6H_5(CH{=}CH)_2]_2CO$	196	198[e]	1.02
Furfuralacetophenone		283	285[c]	0.71

[a] 4-hour reflux. [b] 7-hour reflux. [c] 2-hour reflux. [d] 1-hour reaction at room temperature. [e] 7-hour reaction at room temperature.

In recent years a number of interesting methods have been devised for the identification of aldehydes and ketones, making use of a "quantitative number" approach. Smith and Wheat (*14*) estimated the hydrazine nitrogen of semicarbazones iodometrically by the Jamieson method (*3*). Seaman and co-workers (*11*) described the determination of β-dicarbonyl compounds based on the formation of a copper complex and iodometric titration of the excess reagent after extraction of the complex with chloroform. Veibel (*16, 17*) indirectly made use of a neutralization equivalent by conversion of the carbonyl compound to the *p*-carboxyphenylhydrazone and subsequent titration of the free carboxyl group with standard alkali. Petit (*9*) precipitated the carbonyl compound as a *p*-nitrophenylhydrazone and subsequently reduced the nitro group in the presence of an excess of standard stannous chloride or potassium stannite and titrated the excess stannous ion with standard iodine solution. These methods, though interesting, cannot compare in simplicity with the direct hydroxylamine titration.

EXPERIMENTAL

The experimental procedure adopted in the present paper was modeled essentially on that of Stillman and Reed (*15*). However, because their macromethod used inordinately large samples (1 to 3 grams), a semimicroprocedure has been developed for the present purpose.

Reagents. HYDROXYLAMINE HYDROCHLORIDE. This should be of best quality and contain little or no free hydrogen chloride. The purity of the reagent should be tested by dissolving a small weighed quantity (0.10 to 0.20 gram) in water and titrating with standard alkali to the phenolphthalein end point, and then with acid to the bromophenol blue end point. The alkali titration will show the total hydrochloric acid present, while the acid titration will show the amount of hydroxylamine. These two quantities should be equivalent, or approximately so.

ALCOHOLIC POTASSIUM HYDROXIDE. When required, 3.60 grams of potassium hydroxide are dissolved in 600 ml. of 95% ethyl alcohol (formula 30 may be used).

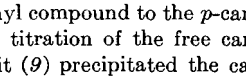
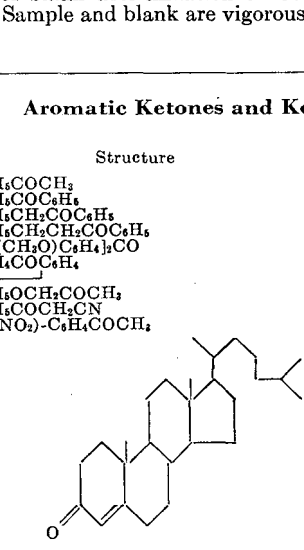
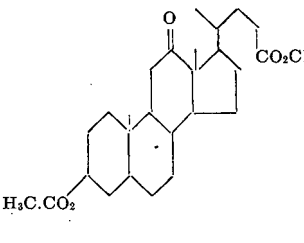
STANDARD HYDROCHLORIC ACID SOLUTION. Approximately 0.05 *N* aqueous hydrochloric acid is carefully standardized by the usual procedures.

BROMOPHENOL BLUE INDICATOR. One-tenth gram of tetrabromophenolsulfonphthalein is ground in a mortar with 3 ml. of 0.05 *N* sodium hydroxide. When solution is complete, the mixture is diluted to 25 ml. with water.

PREPARATION OF HYDROXYLAMINE REAGENT. Nine grams of hydroxylamine hydrochloride are dissolved in 40 ml. of water and the solution is diluted to 800 ml. with 95% ethyl alcohol (denatured, formula 30). To this solution are added, with stirring, 600 ml. of ethyl alcohol containing 3.6 grams of potassium hydroxide and 10 ml. of bromophenol blue indicator. The solution is then filtered through a rapid filter into a 2-liter glass-stoppered bottle for storage.

Analytical Procedure. The sample is accurately weighed (see calculations for method of estimating the size of sample to be used; this depends upon the carbonyl content) and transferred to a 250-ml. "iodine-type" stoppered flask (it is convenient to use such flasks equipped with standard-taper joints to fit a bank of correspondingly equipped water condensers). Because semimicroquantities are being used, great care should be taken in this operation. The more volatile liquids should be weighed in sealed ampoules as described by Niederl (*7*) and crushed below the reagent; solids should be transferred by the usual "charging-tube" technique or by small glass cups, which are used for the less volatile liquids. The size of the sample should be adjusted to yield a titration value between 10 and 15 ml. of the standard hydrochloric acid. Fifty milliliters of the hydroxylamine reagent are pipetted into the flask and the contents are thoroughly agitated. A blank determination on 50 ml. of the reagent is also made. Sample and blank are vigorously refluxed for 1 hour under water-

Table IV. Aromatic Ketones and Keto Steroids

Compound	Structure	Hydroxylamine Number Calcd.	Found	% deviation
Acetophenone	$C_6H_5COCH_3$	466	469	0.65
Benzophenone	$C_6H_5COC_6H_5$	308	295[a]	3.57
Desoxybenzoin	$C_6H_5CH_2COC_6H_5$	286	283.5	0.87
Benzylacetophenone	$C_6H_5CH_2CH_2COC_6H_5$	267	265	0.75
p,p'-Dimethoxybenzophenone	$[p$-$(CH_3O)C_6H_4]_2CO$	232	233[a]	0.43
Fluorenone		311	309	0.65
Phenoxyacetone	$C_6H_5OCH_2COCH_3$	374	370	1.08
Benzoyl acetonitrile	$C_6H_5COCH_2CN$	386	385	0.26
m-Nitroacetophenone	m-(NO_2)-$C_6H_4COCH_3$	340	339	0.29
Cholestenone		150	146	2.73
Methyl-3-acetoxy-12-keto-desoxycholate		123	121	1.65

[a] 7-hour reflux.

cooled condensers, removed, cooled to room temperature under a cold-water tap, and titrated to the acid end point of the bromophenol blue indicator. As discussed under interferences, these experimental conditions will suffice to yield accurate results with a large majority of the carbonyl compounds. However, the presence of certain other functional groups or of steric resistance to oximation requires a modification of the general procedure here presented. This is amplified in the section on Results Obtained and in the discussion.

Calculation. HYDROXYLAMINE NUMBER.

$$\text{Hydroxylamine number} = \frac{(B - T) \times N_{HCl} \times 56.1}{\text{weight of sample}}$$

THEORETICAL VALUE.

$$\text{Theoretical hydroxylamine number} = \frac{(56{,}108) \times \text{No. of CO groups}}{\text{molecular weight}}$$

SAMPLE SIZE.

$$\text{Weight of sample} = \frac{N_{HCl} \times (10) \times (56.1)}{\text{theoretical hydroxylamine number}}$$

Calculated for $(B - T) \cong 10$

where B is the value of the "blank" titration expressed in milliliters of standard hydrochloric acid and T is the sample titration.

Materials Used for Test. The ketones used for testing the method were Eastman Kodak White Label products, used without further purification. The keto steroids were independently prepared.

Stability of Hydroxylamine Reagent. Inasmuch as a solution of free hydroxylamine is unstable, it was considered important to determine the keeping qualities of the hydroxylamine reagent. The reagent was prepared as described above and allowed to stand in a glass-stoppered bottle at room temperature, and 50 ml. were titrated at intervals.

Table V. Variation in Hydroxylamine Number within a Homologous Series for Ketones of Structure R—CO—R'

No. of Carbon Atoms	Molecular Weight	Calculated Hydroxylamine No.
3	58	966
4	72	779
5	86	651
6	100	561
7	114	491
8	128	438
9	142	395
10	156	359
11	170	330
12	184	305
17	254	221
18	268	209

The data obtained are summarized in Table I. The strength of the reagent decreases to about 50% of its original value in 20 days. However, as blank determinations are conducted, this is of little importance. Over a period of 1 week the titer of the solution remains very close to the original.

Results Obtained. The results obtained are summarized in Tables II to IV, inclusive. The values reported represent the average of duplicate and triplicate determinations.

DISCUSSION

The types of keto compounds thus far studied, as exemplified by Tables II to IV, may be classified as simple dialkyl ketones, saturated cyclic ketones, keto esters, keto carboxylic acids, polyketones, aromatic and aralkyl ketones, hydroxyketones, ether ketones, unsaturated ketones, and keto steroids. Their estimation and identification by the determination of their hydroxylamine number are readily accomplished. The types of ketones to which this method of identification may be applied vary from very simple types to very complex substances. The average deviation for the forty-two different compounds studied from the theoretical values is 0.98%. This average deviation from the theoretical makes the identification of a ketone within a homologous series certain because, as may be noted by a study of Table V, very large differences are obtained, especially for values below twelve carbon atoms, and even for very high molecular weight members of seventeen and eighteen carbon atoms the differences (5% between C_{17} and C_{18}) are still beyond the average deviation from the theoretical values. Greater care must, of course, be exercised as the molecular weight increases and the carbonyl content of a compound becomes small. This should be taken so as to obtain at least 10 to 15 ml. of blank minus titration values.

Two general procedures have been developed in hydroxylamine titrimetry of carbonyl compounds, one being to add hydroxylamine hydrochloride reagent to the aldehyde and ketone and then titrate directly the liberated hydrochloric acid (*13*); in the other method free hydroxylamine is used and the excess is titrated after the completion of the reaction. These two procedures are exemplified by the following general equations:

$$RR'CO + NH_2OH.HCl \longrightarrow RR'C{=}NOH + HCl$$

$$RR'CO + NH_2OH \longrightarrow RR'C{=}NOH$$

The latter has been selected as preferable, because standard hydrochloric acid is readily available for titration. The method avoids the difficulty caused by the presence of considerable acidity during the oximation, and the titration error due to differences in pH between the end point shown by the indicator and the true neutralization point of hydroxylamine hydrochloride is eliminated by a blank determination. As shown by Stillman and Reed (*15*), a hydroxylamine reagent comprising an equimolecular mixture of free hydroxylamine and hydroxylamine hydrochloride gives the best rates of oximation and is the reagent employed in the present work. The free hydroxylamine concentration is such as to ensure its presence at all times during the reaction and at the same time eliminates the danger of losing hydrochloric acid from a refluxing mixture of hydrochloric acid and alcohol.

INTERFERENCES

Because the present study is concerned with pure compounds, the interferences are inherent in the compounds themselves and are not of the type which are met with in the analysis of mixtures of compounds or of impure types. The interferences that were actually encountered in the present investigation may be summarized as follows: (1) steric factors, (2) cyclization with formation of isoazoxalones, (3) conjugated unsaturation, and (4) hydrogen bonding. An expected interference due to the presence of carboxyl and carbalkoxy group, such as in keto acids and keto esters, with the formation of hydroxamic acids:

$$RCOOR' + NH_2OH \longrightarrow RCONHOH + R'OH$$

was not met. In order to test this more thoroughly, pure samples of ethyl acetate and ethyl oleate were refluxed over varying periods of time up to 7 hours. In all cases, the blank titration and the sample titration were within 0.05 and 0.10 ml. of each other, indicating that, under the conditions of the analysis used, no hydroximation of the carbalkoxy group takes place to form a hydroxamic acid. As will be noted from Table III, keto esters and keto carboxylic acids show no such internal interference and good agreement between theoretical and experimental hydroxylamine numbers can be obtained.

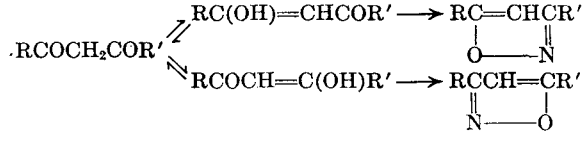
The "steric factor" appears to be a much more serious internal interference and is occasioned by the proximity of bulky groups about the carbonyl group. Diaryl ketones are particularly resistant to oximation, although other factors may be operative. This is illustrated by the data presented in Tables IV and VI. While benzoin is oximated smoothly, the introduction of an acetoxy group materially decreases the rate, and this decrease in rate is very much enhanced by replacing alkyl groups by aryl groups as illustrated by acetophenone and benzophenone. The separation of the aryl and keto groups by a methylene link immediately removes this interference, as may be noted by comparing benzophenone with desoxybenzoin (Table III). However, fluorenone, the cyclic analog of benzophenone, offers no such interference and is oximated smoothly. This is in general true for

Table VI. Interferences from Steric Factors

Compound	Benzoin	Benzoin Acetate	Acetophenone	Benzophenone
Structure	C_6H_5CHOH / $C_6H_5C{=}O$	$C_6H_5CHO_2CCH_3$ / $C_6H_5C{=}O$	$C_6H_5COCH_3$	$C_6H_5COC_6H$
% Oximation				
20 minutes	..	..	101	13.0
1 hour	98.8	66.2	..	83.4
2 hours	..	81.8	..	..
4 hours	..	99.2	..	..
7 hours	..	..	..	95.8

all cyclic ketones (Table II), even those of a complex nature as illustrated by the keto steroids (Table IV).

The interference of cyclization with formation of isoazoxalones is met with in the oximation of the β-diketones. Thus an unsymmetrical β-diketone can give rise to two isomeric isoazoxalones:



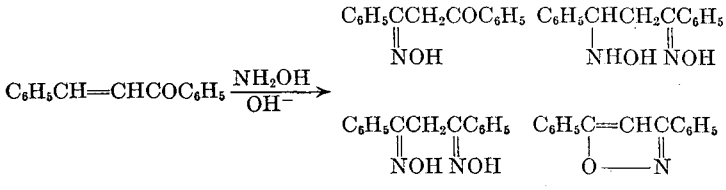
the controlling factor being the extent of enolization of the diketone which, as to be expected, will depend upon the structure of R and R′. The data of Table II indicate that acetylacetone is oximated under conditions in which no enolization is encountered, inasmuch as the expected hydroxylamine number, based on the presence of two carbonyl groups, is realized experimentally, whereas benzoylacetone shows the presence of considerable enolization. However, in spite of this interference, it can be established that the experimental oximation value represents a stable value and thus can be utilized for identification purposes. Thus a measurement of the rate of oximation of benzoylacetone ($C_6H_5COCH_2COCH_3$) under various conditions shows the following:

Reflux, Hours	% Oximation[a]
1, room temp.	58.6
1	59.4
2	59.8
4	49.6

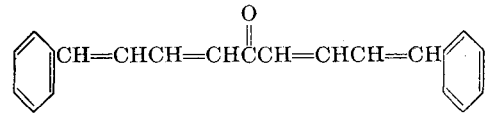
[a] Based on two CO groups.

demonstrating the constancy of its experimental oximation value.

The interference caused by unsaturated conjugation is complex and is due to a number of simultaneous reactions that take place, both at the carbonyl group and with the conjugated system comprising a double bond and a carbonyl group. These reactions depend upon the particular compound and the conditions under which the reaction is carried out. Thus Auwers and Müller (*1*) showed that benzalacetophenone reacts with hydroxylamine in alkaline medium as follows, forming:



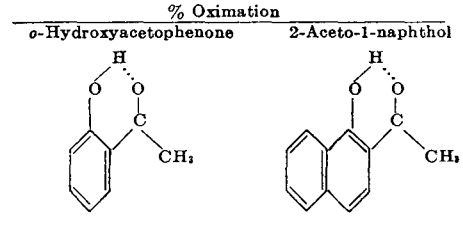
among other products. However, as will be noted from Table III, under the conditions of oximation employed, benzalacetophenone gives excellent agreement between theoretical and experimental hydroxylamine number calculated on the basis of the oximation of the one carbonyl group only. Furfuralacetophenone gives the same result, but a 2-hour reflux period is required. However, in the case of the more highly conjugated systems such as cinnamalacetophenone, dibenzalacetone, and dicinnamalacetone, the Auwers-Müller types of reactions take place under reflux conditions. This can be avoided and the oximation directed solely to the carbonyl group by carrying the reaction out at room temperature; varying times of reaction are required depending upon the particular compound under consideration. However, as in the case of the β-diketones, the higher temperature oximation appears to reach a constant value. This is illustrated by a study of the rate of oximation of dicinnamalacetone



Reflux, Hours	% Oximation
1, room temp.	45.8
7, room temp.	101.0
1	137.3
2	169.2
4	169.2

Thus these highly conjugated carbonyl systems can be approached by either a room temperature oximation or under conditions of reflux.

Interference due to hydrogen bonding could be expected in the aromatic series with the *o*-hydroxy substituted ketones. However, of the types studied (Table III) this has not been realized and has been met with only in the case of 2-aceto-1-naphthol. This is illustrated by a comparative study of the rates of oximation of that compound with *o*-hydroxyacetophenone:



Reflux, Hours	% Oximation: *o*-Hydroxyacetophenone	% Oximation: 2-Aceto-1-naphthol
1	99.3	52.2
2	..	58.2
7	..	100.0

Intermolecular hydrogen bonding, which theoretically could be met with in other types of hydroxyketones, apparently offers no interference to the oximation process.

CONCLUSIONS

The hydroxylamine number is an invariant quantity for a pure compound and can readily be applied to problems in the qualitative and quantitative identification of ketones. It presents an effective teaching aid in courses of qualitative organic analysis. It is applicable to simple and complex ketones and a systematic study of a large variety of different structural types is presented. The keto group in keto acids and keto esters can be estimated without interference by hydroxamic acid formation. Although some β-diketones undergo cyclization to isoazoxalones, they have experimentally constant hydroxylamine numbers which can be utilized for their identification. Interference due to highly conjugated systems can be utilized in the same way, although by conducting the oximation at room temperature the carbonyl group alone is oximated. Hydrogen bonding appears to offer interference only in isolated cases.

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Double-Beam Infrared Spectrophotometer

Chemical and Biochemical Applications

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The value of a versatile double-beam recording infrared spectrophotometer in solving chemical problems is illustrated with several examples. Reproductions of original records show the advantages resulting from high speed, resolution, stability, and flexibility in the measurement of spectra, analysis of samples, and determination of structure. The possibility of compensating for scattering losses in mulls and for solvent absorption in solute study is particularly useful.

IN THE past the utility of infrared spectroscopy in chemical problems has increased directly with the ease of reducing the observations to useful form. Even though the uniqueness of infrared spectra has been known for many years (*6*), the necessity for point readings and curve plotting limited its use to university research and more recently to simple industrial analyses with point reading instruments (*5*). With the advent of commercial automatic recording spectrometers (*4, 14, 16, 17*), infrared spectroscopy became easy and fast enough for general industrial use. The records obtained were useful directly for identification and analysis, but had to be replotted on some absolute scale for easy understandability in conferences and publications. To avoid this labor of replotting, instruments have now been designed to record directly in graphs of uniform wave-length or wave-number scales *vs.* per cent transmittance (*1, 2, 13, 18, 21, 22*). When these features are combined with the high resolution and versatility of modern instruments (*8*) it is possible to record directly a number of types of spectra that would otherwise be difficult and time-consuming to measure.

The purpose of this paper is to show how such an instrument may be applied to a group of diversified chemical problems. Many of them have been done before on single-beam instruments, but without the ease and directness of the present work. Special attention is paid to the advantages obtainable from the use of the double-beam principle under a wide variety of operating conditions. In each case the application is presented as a brief description with reproductions of instrument recordings of per cent transmittance *vs.* wave length. The problems considered and the special features illustrated are:

Type of Problem	Example	Features Illustrated
Structure determination	*o*-Toluidine	Speed, noise level, and scattered light
Spectra of isomers	α- and β-conidendrin	Resolution, compensation for scattering
Product purity	1% ethylbenzene in styrene	Elimination of styrene spectrum by compensation
Steroid spectrum	Dehydroisoandrosterone in chloroform	Elimination of solvent spectrum

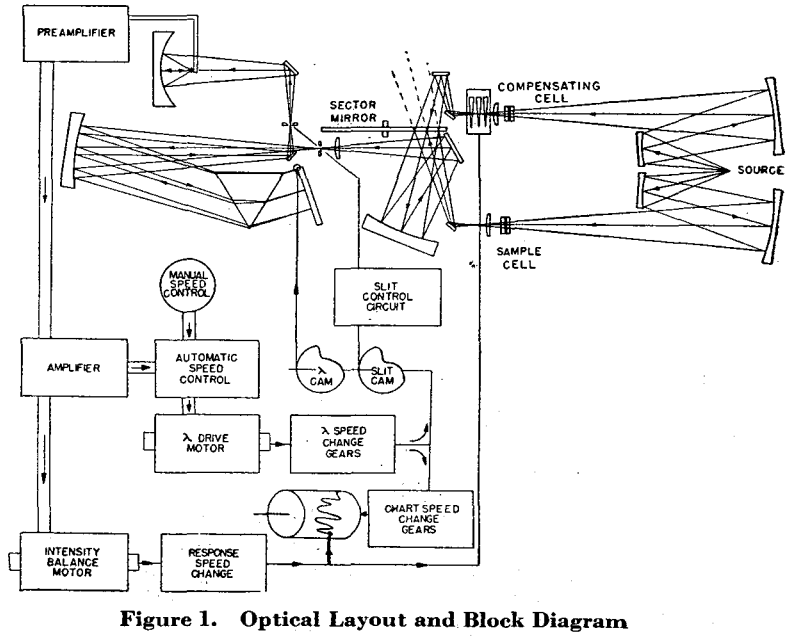

Figure 1. Optical Layout and Block Diagram

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EXPERIMENTAL

The spectra were all measured on the Perkin-Elmer Model 21 recording infrared spectrophotometer. As a detailed description of this instrument has been published (*15*, *20*), only enough is given here to explain its different types of operation. Figure 1 is a schematic optical layout and block diagram, showing how the light goes through the sample and compensating cells and how the different parts of the instrument are related to each other. Figure 2 is a photograph of the spectrophotometer itself.

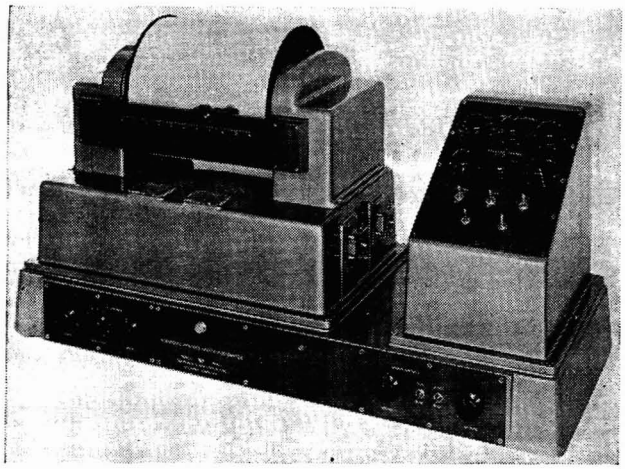

Figure 2. Photograph of Recording Infrared Spectrophotometer

The instrument operates on the principle described by Wright (*22*) in which two beams of light are taken separately from a source and combined by a sector mirror that alternately transmits one and reflects the other into a single direction. One beam passes through the sample and the other through an adjustable wedge aperture and, if desired, a compensating cell. If the intensities in the two beams are equal, their composite formed by the sector mirror has a steady intensity; if the intensities are not equal, the composite beam flickers as the sector mirror moves. The amount of flicker, indicating the degree of off-balance, is detected by the thermocouple, amplified, and used to drive the intensity balance motor in the proper direction to restore balance. The pen records continuously the position of the wedge required for balance. The monochromator used to select radiation of different wave lengths from the composite beam is essentially the same as the one described by Barnes (*4*). Its prism is of rock salt, 60 mm. high and 75 mm. on each face.

The instrument's operating controls are all indicated in Figure 1, except for the amplifier gain and source intensity adjustments. The wave-length scanning speed is manually adjustable mechanically and electrically and also automatically adjustable through a connection to the amplifier that slows down the scanning speed whenever balance is not maintained between the two beams. This makes it possible to scan spectra rapidly in regions where there is no absorption and slowly whenever detailed spectra are present. The degree of this automatic speed suppression is adjustable. The recording drum and the wavelength cam are mechanically coupled together, so that with different change gears records can be made on uniform scales of 1, 2, 5, etc., inches per micron.

The slit cam continuously adjusts the slit widths to give approximately constant energy output from the monochromator between 2 and 15 microns. By changing the resolution control in the slit control circuit, all slit widths may be multiplied or divided by successive factors of the square root of 2. The widest slits are eight times the narrowest. The energy is proportional to the square of the slit width for wide slits, but falls off more rapidly as the slits become narrow. To ensure good operation of the balance motor the amplifier gain control must be readjusted whenever the resolution control is changed.

The response speed control changes the time for full scale deflection of the pen between 4.5 and 110 seconds, thus reducing the noise level of the records when accuracy or high resolution is more important than speed. The corresponding range in root mean square equivalent input noise level is from 10^{-9} volt or 2×10^{-10} watt down to 2×10^{-10} volt or 4×10^{-11} watt.

The liquid absorption cells were of the standard Perkin-Elmer type with rock salt windows and lead amalgam spacers bolted between steel plates. Nujol mulls were made and held between two salt plates. Compensation for scattering in mulls was made with a fine ground salt plate whose transmittance was adjusted by slight rubbing with one finger. For compensating the absorption by solvents a variable space liquid cell was filled with solvent, placed in the reference beam, and adjusted until absorption bands known to belong only to the solvent were removed as completely as possible from the spectrum.

o-TOLUIDINE

The spectrum of *o*-toluidine was measured as a capillary layer between two salt plates. The spectrum shown in Figure 3 was made in 14 minutes per run with 0.2 r.m.s. microvolt for full scale deflection. To show the amount of scattered light, the zero line was measured with an opaque shutter from 2 to 6 microns, a glass one from 6 to 9.5 microns, and a lithium fluoride one from 9.5 to 15 microns. The effectiveness of the grating filter that was used from 7.5 to 15 microns to reduce the scatter is indicated by the fact that 100% absorption of the 13-micron band coincides with the indicated zero line. This spectrum was measured without a compensating plate in the reference beam to avoid interference between the spectrum and the I_0 recorded with the cell removed. The latter indicates the noise level to be expected in the spectrum, the accuracy with which the two beams are balanced over a wide spectral region, and the amount of interference from atmospheric water vapor at this scanning speed without great precautions to dry the inside of the instrument.

α- AND *β*-CONIDENDRIN

The alpha and beta forms of conidendrin are solids with melting points of 255–256° and 210–211° C., respectively. Both samples were measured in the solid state as mulls in Nujol.

In Figure 4, spectrum *A* is the beta isomer as normally measured, including all the scattering losses in the powder. Spectrum *C* is the same sample approximately compensated for scattering losses by a ground salt plate in the reference beam. The curvature of the spectrum at short wave lengths has been pretty well corrected, although the difficulty of matching the scattering accurately makes it unwise to try to measure exact transmittance values under these conditions. The *B* spectrum is that of the alpha isomer, also corrected for scattering. It shows very little similarity to the other. Although both isomers have been as-

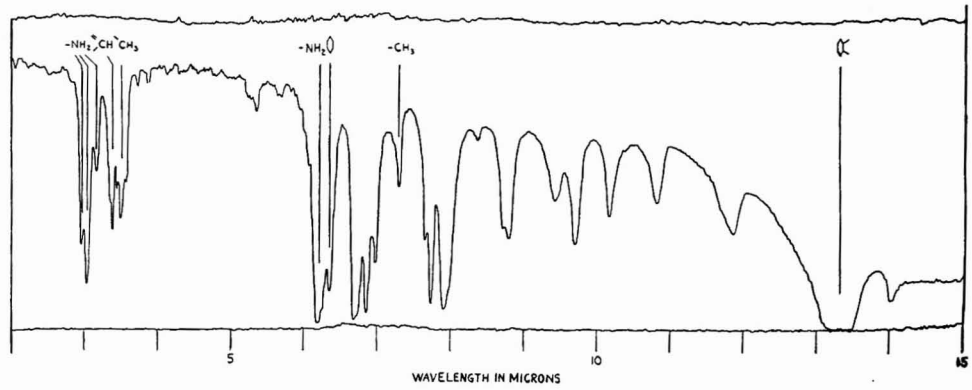

Figure 3. Infrared Spectrum of *o*-Toluidine with Correlations between Absorption Bands and Molecular Groups

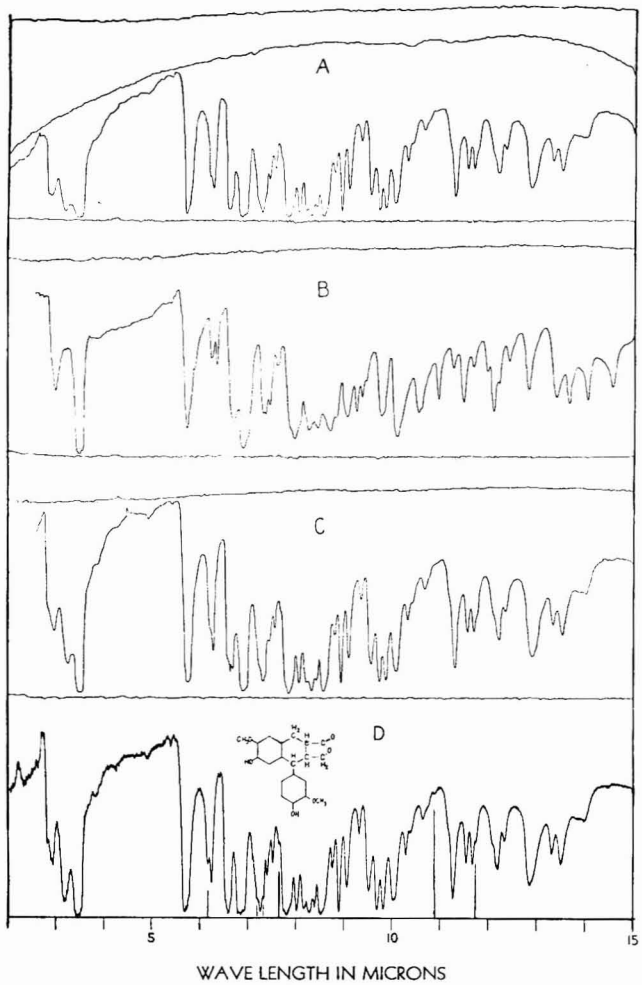

Figure 4. Infrared Spectra of Nujol Mulls of α- and β-Conidendrin

A. β-Conidendrin, uncompensated
B. α-Conidendrin, compensated for scattering
C. β-Conidendrin compensated for scattering
D. β-Conidendrin compensated for scattering, high resolution

signed the same structural formula (*9*, *10*) the differences in their infrared spectra indicate that there must be some marked difference between them. These three records were made in 11 to 12 minutes per run with 0.2 r.m.s. microvolt for full scale deflection. The conditions were identical, except that the source temperature was increased in the compensated runs to help make up for the selective scattering losses at shorter wave lengths, and the gain was increased slightly to compensate for general energy losses. These records have unusually low noise levels and water vapor interference. The zero runs were made with an opaque shutter throughout and without the scattered light filter.

Spectrum *D* is a repeat of *C* at seven tenths the previous slit width with the slowest response speed and a scanning rate of 1 hour per micron. It is especially interesting because it shows additional detail not found in the other record. At the six wave lengths marked on the spectrum, new bands are resolved or clearly indicated. This and the increased separation of the other absorption bands indicate finer structure than is usually observed except in the spectra of light gases. Any reduction in the resolving power of the spectrometer would have resulted in the loss of possible information. This point is emphasized because general statements have sometimes been made that high resolution is not required for liquid or solid samples.

ETHYLBENZENE IN STYRENE

A 1% solution of ethylbenzene in styrene is shown in Figure 5 as an application of the double-beam infrared spectrophotometer to an analytical problem of product purity.

A is a blank run with no absorption cell. *B* is the spectrum of a capillary layer of ethylbenzene. *A* and *B* are both offset compared to the other spectra to make room for *C*, the differential spectrum recorded when the variable cell containing pure styrene was placed in the reference beam. Comparison of the two shows that all the major bands outside the shaded areas were observed in the compensated spectrum. However, when the transmittance in the reference beam fell below 10%, the instrument ceased to operate properly. The bottom pair of spectra are those of the sample, *D*, and of pure styrene, *E*, each measured in the normal manner. The sample is in a fixed 0.1-mm. cell, the styrene in the variable cell set to 99% of the thickness of the sample cell. Because of scratches on the windows of the variable cell, the pure styrene seems to absorb more than the mixed sample everywhere except at 13.4 microns. At this wave length the strong ethylbenzene band reduced the sample's transmittance and caused the two records to cross. The sensitivity was 1 microvolt for full scale deflection, the full scale response time 12.5 seconds, and the scanning time 27 minutes. The filter was used at wave lengths longer than 7.5 microns, as the effects of scattered light increase in proportion to the amount of light absorbed from the reference beam.

DEHYDROISOANDROSTERONE

In recent years there has been considerable infrared work with steroids (*11*) and an active increase is expected because of the recent interest in ACTH and cortisone. The solvent most generally used for this work is carbon disulfide because even in 3-mm. thickness it has extensive transmittance windows. However, the more highly oxygenated steroids are not soluble in carbon disulfide, and chloroform is the best substitute. Unfortunately, in thicknesses greater than 0.3 mm. chloroform has

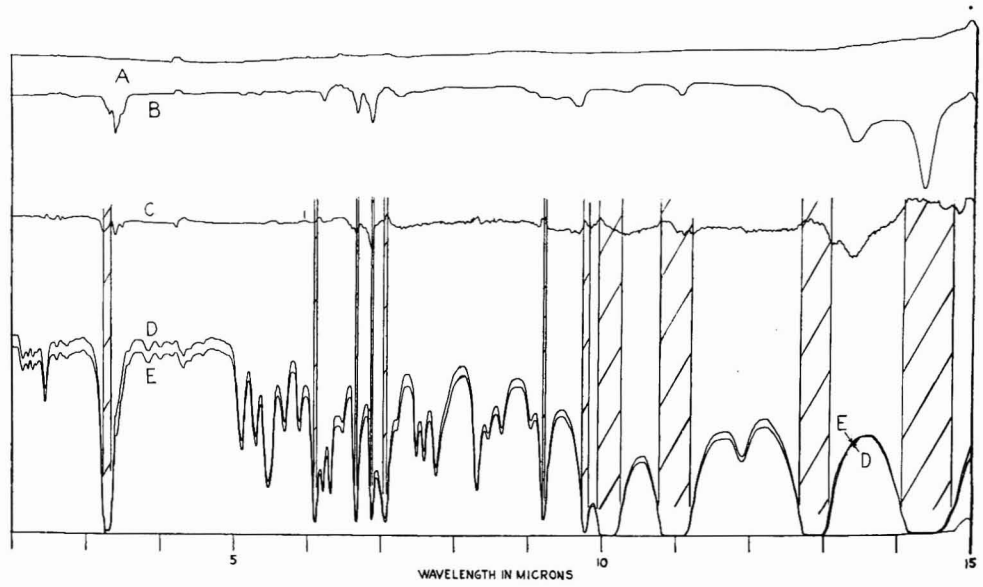

Figure 5. Ethylbenzene in Styrene

A. No sample. *B.* Capillary layer of pure ethylbenzene. *C.* 1% ethylbenzene in styrene, compensated. *D.* 1% ethylbenzene in styrene, uncompensated. *E.* Pure styrene
Cell thickness 0.1 mm.

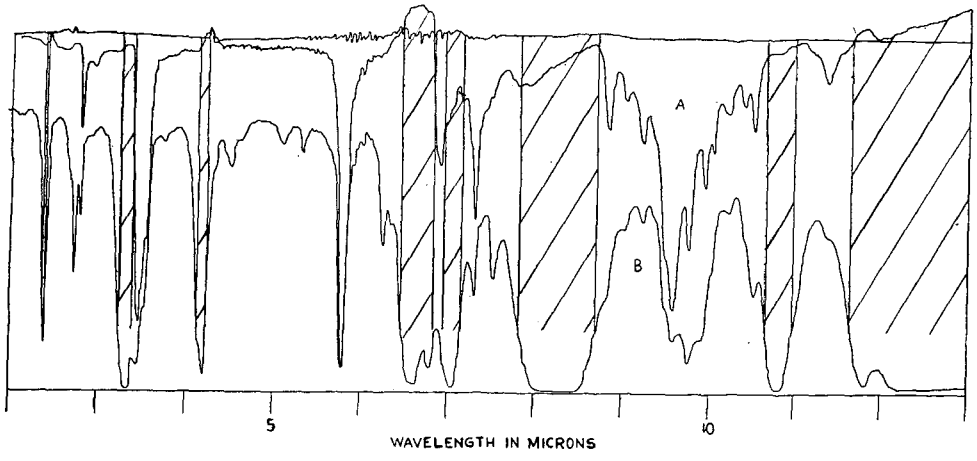

Figure 6. Infrared Spectrum of 1 to 2% Solution of Dehydroisoandrosterone in Chloroform

A. With compensation
B. Without compensation
Cell thickness 1 mm.

so many strong absorption bands that it is not practical without compensation methods to observe the absorption of a small concentration of solute. To illustrate these points, dehydroisoandrosterone was studied at a concentration of 1 to 2% in chloroform.

Figure 6 shows the spectrum of the dissolved sample, *A*, with compensation by an equal amount of solvent in the reference beam and, *B*, without compensation. The thickness of solution was 1 mm. The curves were run in 17 minutes each at a sensitivity of 0.4 r.m.s. microvolt and a response time of 12.5 seconds for full scale deflection. Higher amplifier gain was used for the compensated run than for the others. As the filter was not used, the failure of the 13-micron chloroform band to reach zero indicates the amount of scattered light in the monochromator alone.

The most interesting part of this spectrum lies between 10 and 12 microns, where the sample has a group of characteristic absorption bands. A strong solvent band in this region has been eliminated in the compensated run, presumably to the extent indicated by the removal of the 2.5-micron chloroform band. The resulting spectrum is satisfactory except in the shaded areas, where the solvent's transmittance was less than about 20%. In these regions the instrument was insensitive and drifted slowly up to the right because of a small electrical signal introduced to make the pen responsive at zero transmittance. When these spectra were measured, the humidity was high enough to make the water bands pronounced in the 6-micron region.

SOLVENTS

The spectra shown illustrate the advantages to be gained for chemical research and analysis from the use of a wide range of instrument variables. The principal one is the possibility of extending into the infrared the benefits of solvent compensation that are now realized in instruments for the visible region. Because of the solvent difficulty in the infrared, the art of mineral oil or perfluoro lubricating oil mulls has been developed for study of solid samples. This technique is usually satisfactory for qualitative work but very difficult for accurate quantitative analysis. The experimental technique is difficult, and changes in the crystal form of a compound often change its infrared spectrum. Only one paper (*3*) has appeared describing quantitative analysis in the solid state by use of an internal standard, and it is evident that the method is tedious and requires a skilled technician. Now the use of solvent compensation will permit much more quantitative work with solid samples.

This work, however, requires critical examination of the solvent question. This has already been made for single-beam instruments (*19*) in which any solvent is automatically rejected from general consideration if it has many absorption bands. Except in restricted spectral regions they are, in fact, limited to carbon tetrachloride, carbon disulfide, long-chain paraffins, methylcyclohexane, and a few others. The results illustrated above show that a solvent can be used regardless of the number of its absorption bands in any region where its transmittance is greater than 10 to 30%, depending on the resolution and speed of spectral scanning desired. When examined according to this criterion, many common solvents are useful over most of the 2- to 15μ region, and only limited areas are unusable. It would appear that a number of solvent pairs could be established which (1) are good solvents for a given class of organic compounds, and (2) have complementary transmittance windows. These solvent pairs should be established in the literature with the regions of usability and nonusability designated.

It is desirable that solvent use be standardized, for the spectrum of a solute may vary with the nature of the solvent, and a published solute spectrum may be valid only for the solvent used. For example, a series of very nice correlations of absorption frequency *vs.* position of carbonyl substitution on the steroid nucleus was established (*12*) using carbon disulfide as a solvent. These correlations were observed to shift slightly in carbon tetrachloride, but they were almost entirely destroyed when observed in chloroform (*7*). It seemed that the interaction of the carbonyl group with the polar solvent brought most of the carbonyl frequencies to the same value. Thus in choosing paired solvents, it might be well to select the most nonpolar possible, bearing in mind that double-beam compensation permits solvent thickness much greater than has been previously considered acceptable. It is also apparent that solvent compensation requires the use of an accurate variable-thickness absorption cell.

ACKNOWLEDGMENT

In conclusion, the author wishes to thank V. Z. Williams of the Perkin-Elmer Corporation for help and suggestions in preparing this paper, R. C. Gore of the American Cyanamid Company for samples of *o*-toluidine and α- and β-conidendrin, R. F. Stamm of the American Cyanamid Company for the sample of ethylbenzene, Konrad Dobriner of the Memorial Hospital for the sample of dehydroisoandrosterone, and Norman Wright of the Dow Chemical Company for the sample of styrene.

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BECKMAN FLAME SPECTROPHOTOMETER

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A new flame spectrophotometer, now commercially available, is characterized by a simple detachable atomizer handling samples of 1 ml. or less, a heated spray chamber for evaporating the spray, a versatile burner for oxygen-gas or other flames, and construction of the atomizer-burner unit as an attachment for the Beckman quartz spectrophotometer. Individual measurements can be made in very rapid sequence, and the precision of such measurements is a few tenths per cent of full scale. Interference effects and methods of circumventing them are discussed. Flame spectra and detection limits are given for several dozen elements, and excitation characteristics and methods of analysis are illustrated for a few cases.

FLAME spectrophotometry, as a quantitative analytical technique, has only recently aroused widespread interest in this country. Although flame spectra have been used nearly 100 years for the qualitative identification of elements, the literature discloses little work on the use of flame spectra for quantitative determinations prior to 1929, when Lundegårdh (*7*) published his first treatise on the method. A number of European papers on flame spectrophotometry have appeared since that time, but in this country it was not until 1939 (*6*) that any work was published, and only within the past 3 years has any apparatus been described which differs significantly from that of Lundegårdh.

Lundegårdh and most of his followers employed a spectrographic technique. The solution to be analyzed was sprayed into a flame placed in front of a spectrograph. After exposure, the photographic plates were developed and the optical densities of various spectral lines recorded thereon were measured. With the aid of suitable calibration data the optical densities could be correlated with chemical concentrations with relative accuracies of a few per cent.

The photographic step introduced delay and inconvenience and also limited the accuracy of the method to the reproducibility of photographic emulsions. To overcome the disadvantages of the spectrographic method, direct-reading flame photometers were described as early as 1935. Instruments of this type were made by at least three manufacturers in Germany prior to the war. In 1945 the first direct-reading flame photometer was described in this country (*1*). Since that time many papers have appeared, describing applications and apparatus. Judging from the recent literature, it appears that the apparatus heretofore commercially available leaves much to be desired with respect to convenience and speed of operation, accuracy of measurement, and freedom from spectral interference in multicomponent solutions. To overcome these shortcomings the instrument described in this paper was designed. It has high optical resolving power with attendant freedom from the interference resulting from unresolved overlapping spectral bands. It provides high photometric accuracy. It is simple and fast in operation. Determinations can be made in a few minutes, and no cleaning is required between samples. A single drop of sample is consumed in making a reading, and detectable concentrations may be as low as a few parts per 10^8 for the alkali metals and somewhat higher for other elements. The instrument has already been used for the determination of twenty elements and undoubtedly can be used for many more.

Briefly, the features which distinguish the present instrument, described in greater detail below, from other direct-reading flame photometers, include: (1) a one-piece, high-suction, concentric atomizer requiring no rinsing and providing exceptionally constant and low rate of consumption of sample; (2) a heated spray chamber, which completely evaporates the spray, enhances luminous intensity, and improves stability of performance; (3) use of an oxygen–natural gas flame; and (4) construction of the atomizer-burner unit as a separate attachment to be used in conjunction with the Beckman quartz spectrophotometer whose utility for other purposes is not interfered with by its use as a flame spectrophotometer.

DESCRIPTION OF BECKMAN FLAME SPECTROPHOTOMETER

A satisfactory flame spectrophotometer must have adequate resolving power to differentiate between the spectral emission of the element being determined and the emissions of any interfering substances that may be present. For general utility and precise measurements an essential part of a flame spectrophotometer is necessarily a monochromator which provides a continuous selection of wave lengths with resolving power sufficient to separate completely most of the easily excited emission lines, and freedom from scattered radiation sufficient to minimize interferences. These requirements are provided by the monochromator of the Beckman Model DU spectrophotometer (*4*). As there are many hundreds of these spectrophotometers already in use throughout the world, it was decided to design a flame spectrophotometer which could take advantage of this monochromator and the sensitive and accurate photometric equipment of these instruments. In a paper presented in November 1947 before the Soil Science

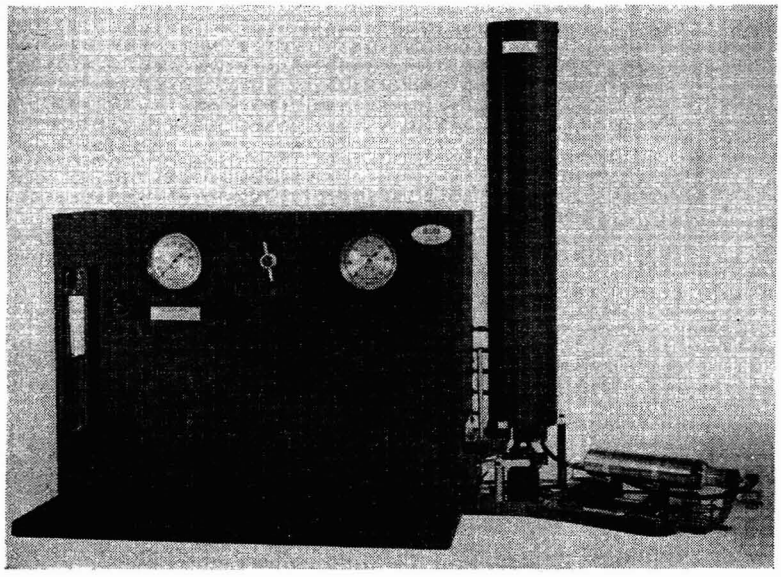

Figure 1. Flame Spectrophotometer Accessory

Society of America, Rogers (*9*) told of his use of the Model DU instrument with an air-acetylene flame for the routine determination of potassium and calcium in soils. Others also have used the instrument for emission photometry.

General construction of the Beckman flame spectrophotometer accessory is shown in Figure 1.

It consists essentially of a burner and a spray atomizer, with associated gages and regulators for air, gas, and oxygen. The components are assembled in a unit which provides a mounting for the Model DU spectrophotometer. The control box on the left contains the pressure gages, control valves for air, gas, and oxygen, and a pressure regulator and filter for the air. The water-cooled burner (Figure 5) is designed for natural gas and oxygen and produces a broad steady flame which does not require precise optical alignment with the monochromator. The fuel is burned at many small ports, which are located around larger holes through which the air stream containing the atomized sample is introduced. The products of combustion pass upward through a water-cooled chimney. The sample is contained in the 5-ml. beaker at the extreme right (Figures 1 and 6). The capillary inlet tube of the atomizer (Figure 6) which dips into the sample has its inlet orifice sharply tapered to about 70 microns to prevent entrance of solid particles that might clog the capillary. With air at 10 to 30 pounds per square inch pressure flowing through the atomizer, a suction of about 1 to 3 meters of water is created. The effect of change in level of the sample is, therefore, entirely negligible. The atomizer and spray chamber are made of borosilicate glass and the burner is constructed of nonreactive materials to avoid contamination. The spray tip, which is concentric with the air nozzle, has an internal diameter of about 100 microns and produces a very fine and constant spray which evaporates completely before striking the walls of the spray chamber. Condensation on the walls of the spray chamber is prevented by an electric heater wound on the spray chamber body, and the solute passes completely into the flame in the form of dry microscopic particles. The burner operates normally with natural gas at a pressure of 1 to 3 inches of water and oxygen at a pressure of 15 to 50 inches. It consumes about 15 cubic feet of oxygen per hour.

Figure 2 shows the flame photometer with the Model DU spectrophotometer in place. After the initial installation of a 10,000-megohm phototube load resistor to increase the sensitivity fivefold, it is merely necessary to detach the lamp housing from the Model DU instrument to change from absorption to emission spectrophotometry.

FLAME CHARACTERISTICS

The oxygen-gas flame was selected because of its high temperature and convenience. An air-gas flame, because of its lower temperature, is suitable only for the determination of elements which are easily excited, such as the alkali metals. Oxygen-acetylene and oxygen-gas produce much hotter flames and correspondingly greater excitation. The former is somewhat superior in this respect to the latter, but this advantage is offset by the higher level of background illumination and the more stringent requirements imposed upon the design of a burner which will produce a steady, quiet flame with an oxygen-acetylene mixture without danger of flashing back. Mixtures of oxygen and artificial gas containing much hydrogen and carbon monoxide also flash back rather readily because of the high flame propagation velocity. Satisfactory fuels having relatively low flame velocity include natural gas and bottled propane and butane (cf. Table I, B).

Dilution of the flame by the air used to spray the sample reduces both the background illumination and the intensity of metallic excitation, and the ratio of spray to flame must be taken into account in the design of a flame photometer. Whereas tank nitrogen can be used instead of air for the atomizer, its use entails a not inconsiderable loss of sensitivity and increases oxygen requirements of the flame. On the other hand, oxygen can be used advantageously to atomize the sample. The flame is thereby altered in the direction of greater background intensity, but at the same time the intensity of metallic emission is roughly doubled. Whereas the present instrument is designed for an oxygen–natural gas flame with air for spraying, it can be used equally well with the following flames when oxygen is used for atomizing: natural gas, with very little additional oxygen; air-acetylene; artificial gas with a little air; and hydrogen alone.

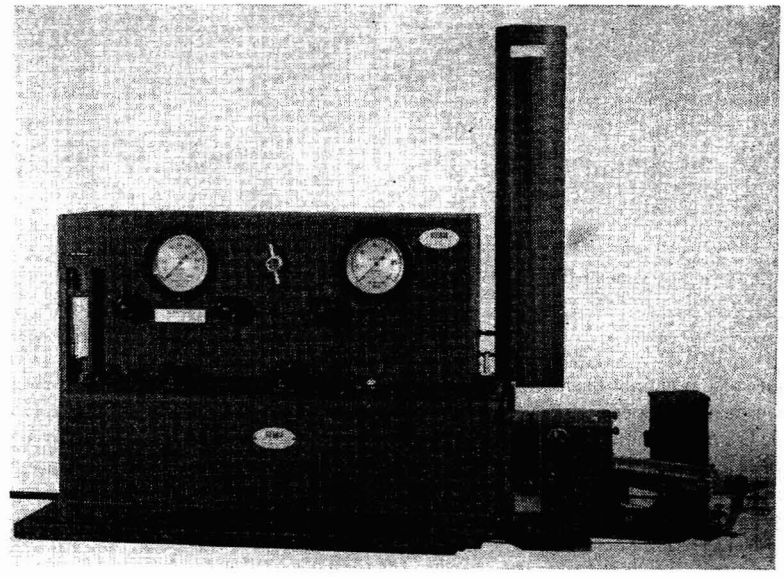

Figure 2. Complete Flame Spectrophotometer

The hydrogen flame is particularly desirable because it eliminates one of the required gases (it replaces compressed air and piped or bottled gas with a tank of hydrogen, the oxygen tank being connected to the atomizer instead of to the burner directly),

Table I. Examples of Data for Various Types of Determinations

A. Rapid determination of sodium in water (4 to 6 readings per minute). Natural gas, heated spray chamber. Readings taken in triplets, on first standard, unknown, and second standard, successively

Standard, 42.3 P.P.M. Na	Unknown	Standard, 92.0 P.P.M. Na
70.3	76.1	108.3
70.5	76.8	107.4
70.1	75.6	107.1
70.2	76.5	107.6
70.4	76.2	...
Av. 70.3	76.2	107.6

Standard errors of ratios = 0.4, 0.5%
Graphical interpolation (Figure 7) yields 49.1 p.p.m. Na for unknown
Probable error of result = 0.2% or 0.1 p.p.m.

B. Readings on sodium in tap water, using oxygen-butane flame

First Series		Second Series	
101.1	100.4	101.5	101.3
100.7	100.7	101.5	101.7
100.8	101.5	101.2	101.6
101.5	100.5	101.4	101.2
101.5	101.4	101.8	101.4
101.2	101.0	101.6	101.5
Av.	101.0		101.5
Standard error %	0.40		0.19

C. Determination of sodium in water, using hydrogen flame and oxygen atomizer

	Blank (Water)	Standard, 50 P.P.M.	Standard, 100 P.P.M.	Unknown
	0.5	68.0	98.5	105.6
		68.0	98.6	105.2
		67.9	98.5	105.5
		68.0	98.5	105.8
		68.0	98.5	105.4
	0.5	67.8	98.4	105.4
Av.	0.5	67.95	98.5	105.5
Net reading		67.45	98.0	105.0
Ratios		1.453	1.072	
Ratios for gas flame		1.458	1.072	

Sodium found by graphical extrapolation (Figure 7) 114.2 p.p.m.

D. Determination of traces of sodium in distilled water. Part of longer sequence of measurements. Instrument at maximum sensitivity; shutter left open continuously, dark current not adjusted. Hypothetical sodium free water (which was not available) would depress flame background by 0.2 unit, as determined by measurements at adjacent wave lengths

Water Sample	Readings: Flame	Readings: Sample	Readings: Flame	Net Reading Corr. for Background	Corrected for Drift	Na Found, P.P.M.[a]
A	5.9	30.4	6.1	24.6	24.6	0.163
B	6.4	18.6	6.3	12.5	12.5	0.083
B	6.8	19.3	6.8	12.7	12.8	0.085
A	...	45.7	7.3	38.6	38.8	[b]
A	7.7	31.8	7.8	24.2	24.4	0.162
A	8.7	32.7	8.7	24.2	24.4	0.162

[a] Calculated from calibration curve which was found linear at these concentrations.
[b] Obviously contaminated by accidental Na.

E. Determination of potassium in brine containing 19.58% K as KCl by gravimetric assay. Standard prepared to approximate sample, containing KCl, borax, Na_2CO_3, and NaCl. Natural gas–oxygen flame, unheated spray chamber, propyl alcohol mixed with sample and standard

KCl Found, %	
First sample	Second sample
19.73	19.67
19.47	19.63
19.39	19.65
19.59	
19.57	
Av. 19.55	19.65
General av., % 19.59	

F. Determination of calcium in water, in presence of sodium. Instrument at high sensitivity. Heated spray chamber. Wave length 554 mμ. Both sample and standards contained 102 p.p.m. Na

Water Blank	Standard, No Ca	Sample	Standard, 1.00 P.P.M. Ca	Ca Found, P.P.M.
9.2	11.4	13.0	15.1	0.43
9.5	12.1	13.4	15.6	0.37
8.6	11.4	13.3	15.2	0.50
9.7	12.0	13.7	15.7	0.46
				Av. 0.44

G. Determination of copper in methyl violet (measurements made by Marshall Odeen). Isopropyl alcohol used as solvent. Sample contained 1.75% (w/v) of dye; standard contained 5 p.p.m. Cu (w/v). Blank, pure isopropyl alcohol. Wave length 324.8 mμ. Natural gas–oxygen flame adjusted to give same reading with blank as with nothing, in order to eliminate possible background effects

Blank	Standard	Sample	Net Standard	Net Sample
99.8	104.6	101.1	4.8	1.3
100.0	104.8	100.9	4.8	0.9
100.2	104.9	101.0	4.7	0.8
100.1	105.1	101.4	5.0	1.3
100.5	105.4	101.3	4.9	0.8
100.2	104.7	101.2	4.5	1.0
Av. 100.1	104.9	101.1	4.8	1.0

Copper found 0.006% of methyl violet

because it cannot flash back, damage the burner, or produce soot, because it is perfectly quiet and remarkably steady (see Table I, C), and because it provides the greatest ratio of metallic emission to flame background over large regions of the spectrum. Whereas the hydrogen is consumed at a rate of about 40 cu. feet per hour in a typical instrument, and oxygen at about 25 cu. feet per hour, both gases can be conserved by turning them down to very low pressures between runs, without extinguishing the flame.

It is felt that the versatility of the instrument in being equally adaptable, without alteration, to any of a variety of gaseous media is a real advantage.

Typical flame background spectra are shown in Figure 3. Relative photocurrents are plotted against wave length for the natural gas–oxygen flame (atomizer air being turned off) and for the hydrogen flame with atomizer oxygen flowing. With slit widths of 0.1 and 0.2 mm. the OH spectrum in the ultraviolet and the molecular water bands in the infrared are partially resolved. The sharp peaks at 306 to 315 mμ interfere with very few metallic lines, and the same is true of the water peaks near 960 mμ.

It is essential that the flame burn steadily without undue care in the adjustment of controls. The burner has been carefully designed to achieve this result. Gas flow is adjusted with a needle valve, pressure being read on a liquid manometer. No gas pressure regulator is provided, because regulation was found to be superfluous under proper operating conditions where the luminosity is at a broad maximum or plateau with respect to gas and oxygen pressure. Figure 4, for example, shows relative photocurrents for the lithium line at 671 mμ as a function of oxygen pressure for various gas pressures. The broad maxima are clearly evident. Other substances give similar results. Pressure adjustments are not critical and fluctuations of pressure have not been troublesome. Suitable pressures are readily found experimentally by setting the wave-length scale for a particular element and adjusting the gas and oxygen pressures until maximum brightness is obtained. Thereafter the adjustments remain fixed and can be preset on future occasions.

With the hydrogen flame similar conditions prevail. A broad maximum in the luminosity curve will be found by adjusting either the hydrogen or the atomizer oxygen pressure. Near the optimal adjustment a change of 1% in the oxygen pressure affects the intensity of sodium light by 0.05 to 0.1% and a change of 1% in the hydrogen pressure affects it by about 0.1%. Actual maxima with respect to both hydrogen and oxygen cannot be achieved simultaneously, and the above figures represent a compromise adjustment. With good commercial regulators on the tanks, changes greater than those mentioned are unlikely during an analysis.

The pressures which produce maximum brightness vary somewhat, in a gas flame, depending upon the element, its concentration, and the wave length used for measurement, but the variation usually is of slight significance, for it is found in practice that

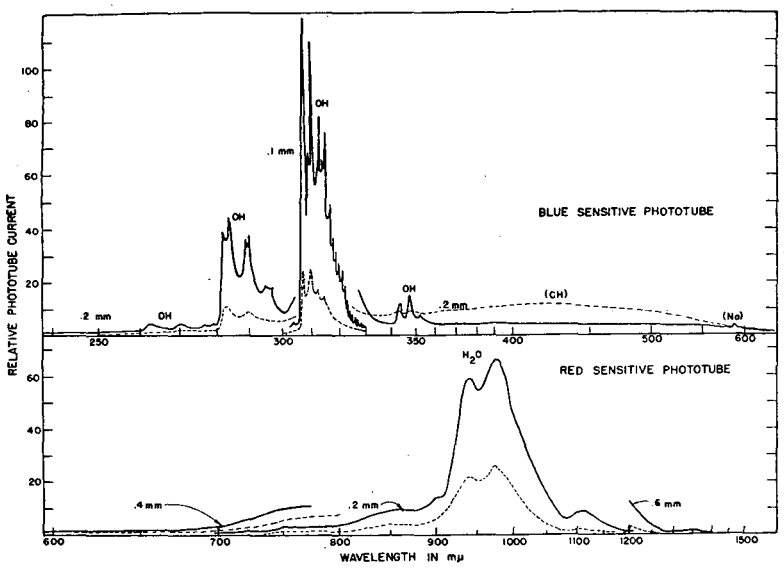

Figure 3. Flame Background

Emission of natural gas–oxygen flame with atomizing air off (- - -) and hydrogen flame with atomizing oxygen on (——). Slit widths shown as parameters. Molecules responsible for band peaks indicated

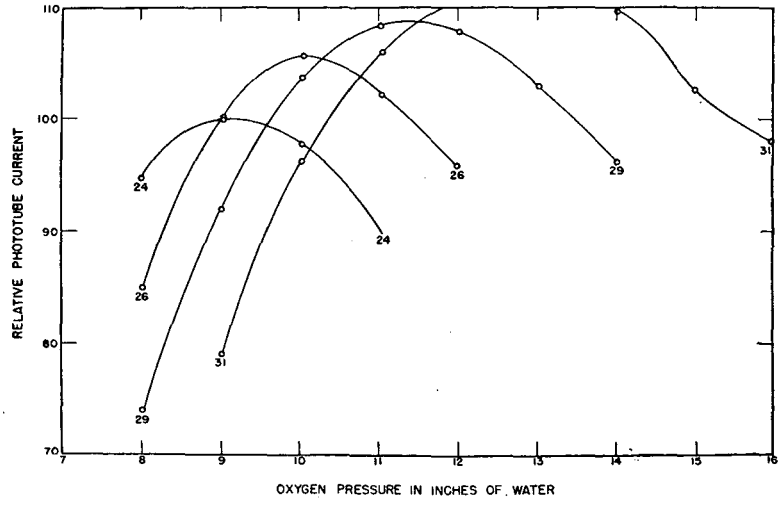

Figure 4. Lithium Emission at 671 Mμ

Parameters are gas pressure, mm. of isopropyl alcohol manometer fluid

the same settings can be used for practically all determinations. Failure to operate at maximum brightness does not directly affect the accuracy of analysis. It is desirable to operate at or near the maximum brightness because the narrowest spectral band widths can be used (important when nearby lines of other elements may cause interference) and because at maximum brightness the flame burns most steadily, with minimum flicker and least disturbance by fluctuations in gas and oxygen pressures.

The construction of the burner used is shown in Figure 5.

ATOMIZER DESIGN

The most exacting problem in flame spectrophotometer design is presented by the atomizer. This deceptively simple little device must introduce the sample into the flame at the maximum stable and reproducible rate. It must remain unattacked by corrosive solutions and be rugged and easily cleaned. It should preferably be easily altered or interchanged to accommodate samples of varying viscosity and surface tension. The atomizer and spray chamber used are shown schematically in Figure 6.

Experience with reflux-type atomizers, which permit the spray to hit the walls of the chamber and return to the sample, demonstrated that drifts are virtually unavoidable and of such magnitude as to set a severe limit on instrument accuracy and convenience. The drifts result from temperature and concentration changes caused by progressive evaporation, either of the sample or of wash water in air prehumidifying and washing towers. It is also difficult to prevent sample film on the walls from reaching the flame, and to avoid contaminating subsequent samples. Sprayers of designs which permit the spray to hit the walls of the chamber without returning to the sample consume excessive quantities of sample or tend to be erratic, or both.

The solution to all these problems was found in an atomizer in which the spray dries completely before the air stream is deflected from a free straight path. As there is no precipitation or condensation on the walls of the chamber, no cleaning is required when changing samples. The spray chamber is swept completely clean within a few seconds after removing a sample. Readings on different samples can therefore be made very rapidly (3 to 6 samples per minute; cf. Table I, A). Furthermore, the volume consumed per reading may be as small as one drop (0.05 ml.), while the total volume may be as small as 0.2 ml. A sample of 2 to 5 ml. is usually sufficient for accurate determinations of several constituents.

The adopted atomizer design shown in Figure 6 has a high suction pressure differential (over 100 cm. of water head), making the sample introduction rate relatively independent of height of solution in the sample cup. It is also easy to overcome plugging, because the limiting orifice is made by breaking off a drawn glass tube tip of rapidly diminishing cross section, located at the inlet end of the tube. The heavy-walled air nozzle surrounds and protects the spray tip, so that

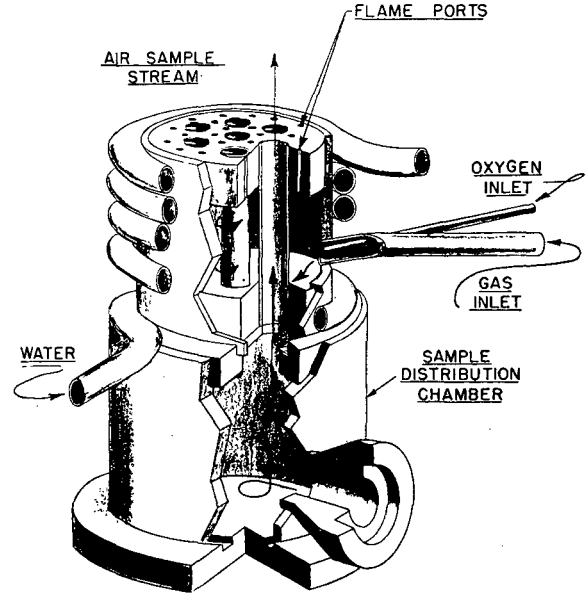

Figure 5. Cut-Away View of Burner

by merely stopping the air nozzle with the finger air is forced out of the orifice in reverse to blow it out.

Changes in viscosity and surface tension affect the performance of any atomizer. The effects of differences in viscosity and surface tension of samples often can be reduced by operating at higher dilutions to bring these properties of the solution nearer those of the solvent, within the limits set by the sensitivity of the instrument. Here the high photometric sensitivity and continuously adjustable slit of the Model DU spectrophotometer are especially advantageous, for the controls permit a millionfold range in full-scale luminosity. The most intense radiation that can be measured exceeds the faintest detectable by 10^7 to 10^9, depending on the sharpness of the spectral line or band. Thus determinations of major and trace constituents or of easily and difficultly excited spectra may often be made at a single sample strength, or, for routine analyses, conditions where disturbing effects are minimized may be selected.

A dry spray atomizer is difficult to construct so that it delivers an adequate concentration to the flame. This requirement may be satisfied by lowering the surface tension of samples by carefully controlled additions of about 20% of propanol or other organic solvent (cf. Table I, E), but after extensive trials it was decided instead to ensure complete evaporation by heating the spray chamber. The 70-watt heater employed can be left connected to the power line while changing samples or adjusting the instrument, and it is not critical as to voltage. Its use makes atomizer design and adjustment much less critical in attempts to obtain maximum spray rate without objectionable fluctuations in flame brilliance.

Solutions in organic solvents of low viscosity can often be used directly in atomizers (cf. Table I, G). When oxygen is used for spraying, one should be certain that explosive mixtures are not formed in the spray chamber. Other organic materials can be prepared by customary wet- or dry-ashing procedures. Typical atomizers exhibit a reproducibility (standard error) of 0.4% or better for individual readings and 0.1% for the average of several readings (see Table I, A, B, and C). As readings may be taken rapidly, averaging several readings is not time-consuming and when interspersed with control and blank readings offers insurance against errors due to drift or excessive pressure fluctuations. The photometric precision of the spectrophotometer is 0.1%, so that an analytical accuracy of 0.2% of the amount present in the sample is entirely feasible for many elements. This approximately equals the analytical accuracy of absorption measurements with the same instrument, and is more than tenfold better than the best spectrographic accuracy.

INTERFERENCE EFFECTS

Interferences often are the most troublesome aspect of flame photometry, and are the more hazardous to analytical accuracy, because they may exist unsuspected. The etiology of six distinct types of interference and procedures for evaluating or minimizing them are discussed in the numbered paragraphs below.

A few experimental studies of interferences with the Beckman flame spectrophotometer have been published, and others are expected to appear soon. A detailed procedure for the accurate determination of sodium and calcium in water has been presented (*5*), wherein the extent of interferences of types 1 and 5, below, is described for the ranges 0 to 200 p.p.m. of sodium and 0 to 20 p.p.m. of calcium and the method of circumventing them is shown.

Mosher *et al.* (*8*) describe the determination of sodium and potassium in plasma and urine, including effects of instrumental adjustments and of air and oxygen pressures. Their standard solutions included sodium, potassium, calcium, and magnesium chlorides, ammonium phosphate, glucose, urea, gelatin, cholesterol, and alcohol. They show the results of recovery studies on sodium and potassium in plasma, in which the errors averaged 1 to 2%, and of viscosity studies involving gelatin. Sodium in the concentrations dealt with enhanced the potassium light by 8%.

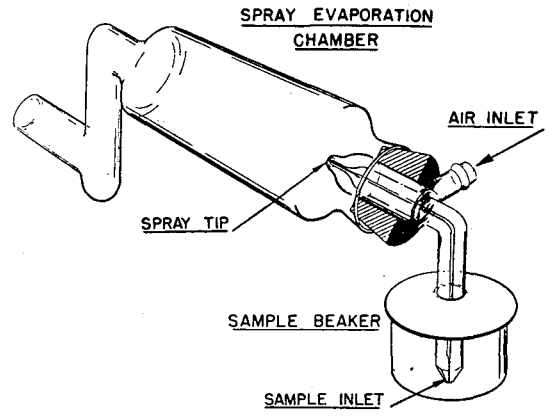

Figure 6. Atomizer Construction and Relation to Spray Chamber

Brown *et al.* (*3*) discuss the determination of calcium and magnesium in leaves. Influences of sodium (with and without a didymium filter), potassium, and acetic and nitric acids on the calcium light are shown. Linear calibration curves were obtained with magnesium at 371 mμ and calcium at 626 mμ. They report that routine analyses could be completed in 2 minutes per metal and that comparison of the photometric with chemical analyses showed standard deviations of 3 or 4%. High and low contents of potassium, sodium, and chloride in the leaves had no important effect. In connection with magnesium, a hydrogen flame has been found to give considerably improved sensitivity, especially at 371 mμ, where convenient instrumental settings will permit of reading easily to 1 p.p.m. of magnesium. Interferences by sodium, potassium, and calcium are also decidedly smaller than in the natural gas flame, and it is considered feasible to determine magnesium routinely, using the hydrogen flame, in biological fluids.

Variations of the apparent or actual emission of radiant energy by excited atoms or by molecules or radicals containing atoms of the species sought may arise in the following ways.

1. **Such mechanisms as quenching, sensitization, reabsorption (reversal), and interference with combustion processes,** resulting in altered flame temperature, may cause alteration of the energy in an emitted line or band by other substances in the flame. In the instrument described here, whereby observations are made in relative terms, we are concerned only with the effects resulting from disparities between the composition of samples and comparison standards. It has been found that instrumental operating conditions can be kept sufficiently uniform during the short time required for a series of observations to make instrumental drifts and random fluctuations practically negligible, although, as in any well designed device, they set the ultimate limits on available accuracy.

Many of the effects described below have been improperly ascribed to direct interference by one substance on the emission intensity of another. However, after all other sources of interference have been carefully examined and eliminated or allowed for, there sometimes remain substantial direct interference effects. Fortunately, their magnitude is often approximately proportional to the concentration of the interfering substance, and their relative importance can therefore be reduced by merely diluting the solution to the point where other sources of error, such as flame background intensity, become of equal importance. Where this expedient does not render them negligible it often reduces them to such an extent that they are easily compensated by determination of the concentration of the interfering substance (where feasible, by flame photometry on the same sample), and allowed

for by computation or by preparation of a more representative comparison standard.

When working with samples that are not of a routine nature, and interference effects are suspected or feared, a simple and practical procedure is to compare the apparent concentration of the element in question in the undiluted sample with that in a portion diluted to half its original concentration, using a suitable pair of standards having a concentration ratio of 2 to 1. In the absence of interference, the second value will be exactly half the first. If this is not the case, a first approximation to the corrected reading on the second sample will be twice the second reading minus half the first. Further dilutions, if they do not entail loss of full-scale precision, can improve the certainty of the interference estimate.

A hypothetical example of this procedure would be the following:

Let Solution I contain 100 p.p.m. of metal A and 100 p.p.m. of metal B, which at this concentration increases the intensity of emission of A by 10%. Furthermore, assume that the intensity of emission of A is directly proportional to its concentration in this range, and that the absolute increment of emission by A due to the presence of B is proportional to the product of their concentrations, which as indicted above is commonly approximately true.

Let Solution II, the standard, contain 100 p.p.m. of A only. Comparing I against II in the flame photometer, I will appear to contain 110 p.p.m. of A. Now with the purpose of detecting interference, dilute Solutions I and II with equal volumes of solvent, forming Solution III (50 p.p.m. of A and 50 p.p.m. of B) and Solution IV (50 p.p.m. of A). At 50 p.p.m., B will enhance the emission of A by only 5%. Therefore, Solution III, compared against Solution IV (known to contain 50 p.p.m. of A), will appear to contain 5% more than 50 p.p.m., or 52.5 p.p.m. The fact that 52.5 is less than half of 110 indicates that an interference is present. To arrive at a corrected value for the concentration of A in III, take $2 \times 52.5 - (1/2 \times 110)$, which is 50. Because the simplified assumptions applying to interferences were true in this hypothetical case, the result is exactly correct.

The following is an actual case involving a common interference, that of sodium with calcium:

Solution	Na, P.P.M.	Ca, P.P.M.	Ca, Reading	Apparent Ca
I	100	10	48.2	9.64
II	0	10	50.0	(Standard)
III	50	5	24.6	4.92
IV	0	5	25.0	(Standard)

Fortunately, the calcium light is exactly proportional to concentration (Figure 9) but sodium exercises a complex type of interference (see paragraph 5 below) upon calcium. However, the fact that the diluted sample appears to contain more than half as much calcium as the undiluted sample leads one to apply the rule for finding a first approximation: $2 \times 4.92 - (1/2 \times 9.64) = 5.02$, and so the original sample, in the absence of other information, would be surmised to contain 10.04 p.p.m. of calcium. The true value, if greater accuracy is required, could be obtained in this case by measuring the sodium (which is not interfered with by calcium) and then employing a standard containing the same sodium content as found in the sample. The same anions should be employed in the solutions under comparison, inasmuch as differences in emission due to different anions cannot be assumed *a priori* to be negligible.

An example of the determination of a trace of calcium in the presence of 200 times as much sodium is given in Table I, F.

2. **Energy at other wave lengths** than those intended to be measured may reach the photodetector. It is too obvious to require further elaboration that the monochromator must be capable of excluding emission at wave lengths adjacent to or remote from the selected band by substances uncontrolled in the comparison standards. Naturally, this problem is simplified if the photomeasuring equipment is sufficiently sensitive to permit the monochromator to be used at slit widths approaching those at which the resolution is limited only by the optical design of the monochromator. In the ideal monochromator this limit is proportional to the dispersion of the dispersing element (prism or grating) and is set by diffraction effects at the slits. In realizable instruments optical aberrations and slit image curvature (which can be matched by a practicable slit mechanism at only one wave length) introduce additional deviations which add to the Rayleigh limit slit widths. The design features of the Beckman Model DU monochromator have been described (*4*).

In flame photometry a higher premium must be set on photodetection sensitivity as compared with absorption photometry because line emission is confined practically to single wave lengths. The line energy passed by the monochromator is therefore proportional to the first power of the slit width, whereas background emission and scattered radiation are proportional to the second power. The advantages in interference problems of being able to work at high dilution and the use of a high temperature flame with its associated inherent high background radiance emphasize the desirability of high detection sensitivity. These are the reasons for modification of the photodetection system by increasing the phototube load resistor fivefold to 10,000 megohms, despite some loss of response speed and a slight decrease in linearity and stability.

3. **Direct interference by (nonbackground) radiation** can occur when a desired line falls within a molecular emission band. When emission bands actually overlap, interference cannot be obviated by increased resolution. However, instrumental versatility will often provide an easy solution. An example would be a sample containing barium and lanthanum. The barium emission at 515 to 550 mμ would be convenient to use were it not for the lanthanum (oxide) bands which interfere. Conversely, unresolved barium bands interfere with the lanthanum band at 563 mμ. Here the solution lies in selection of other spectral regions where mutual interference does not occur. The lanthanum peak at 798 mμ and the barium emission at 830 or 873 mμ are much less subject to mutual interference and are more sensitive than the green bands.

Even if overlap cannot be avoided, it will usually be found that multicomponent analyses by methods similar to those used in infrared absorption spectrophotometry (*2*), where interference is the rule rather than the exception, will prove usefully convenient and accurate.

4. **Intense adjacent line emission** can also cause direct interference. Sometimes an extremely brilliant atomic emission may interfere with the measurement of a neighboring line, either through Doppler broadening or because scattered radiation tends to be concentrated near the line image. An example is found in the determination of boron in borax. The boron band at 548 mμ cannot be used because of the overwhelming brilliance of the sodium *D* doublet from solutions sufficiently concentrated to permit determining boron to 1% of its amount. The boron bands at 521 and 495 mμ, however, lie well away from the dazzling sodium light and, although weaker than the 548 mμ band, are sufficiently bright for accurate determination of boron.

These procedures for avoiding interference errors by selecting wave lengths free from interference emphasize the inadequacy of filter photometers which are limited to a few, fixed, broad pass bands. The ability to select any desired narrow wave-length band over a wide range is of prime importance. A large proportion of useful wave lengths are shorter than 400 mμ, while the near infrared is particularly useful for the determination of such metals as rubidium, cesium, barium, lanthanum, and strontium.

5. **A general increase of flame background at all wave lengths** may occur, and is common with materials containing sodium and potassium. It extends over most of the spectrum, but is not due to scattered light, as may be shown by interposition of a didymium filter, which cuts out the sodium line almost completely but leaves unaltered the background increase due to sodium at other wave lengths. This effect may be of such magnitude as to require a careful preliminary study, in the event that small amounts of any element are to be determined in the presence of considerable alkali metal and dilution has already been carried

to its practical limit. Even magnesium at 285 mμ is subject to this influence by sodium; a sodium concentration of 0.1% will increase the background light at 285 mμ by an amount equivalent to 10 or 20 p.p.m. of magnesium under conditions which in the absence of sodium would permit readings to be made to 1 p.p.m. or less. Similarly, the determination of calcium in the presence of 100 times as much sodium necessitates rather large corrections for both background change and depression of the calcium light proper—effects which though of opposite sign do not by any means cancel each other (see Table I, F). Much potassium in a sample likewise makes difficult the detection of traces of lithium or rubidium, but a careful scanning of the region in the immediate vicinity of the lithium or rubidium lines will reveal the presence of any actual emission by these metals. In practice, such background corrections can be either measured or obviated by use of a blank containing the proper amount of interfering metal but none of the metal being determined.

6. **Spray rate alteration,** by uncontrolled constituents or by conditions (especially the temperature), which affect the hydrodynamics of the atomizer, is a common and frequently misinterpreted cause of interference. Both the viscosity and the surface tension of the solution are of first importance in determining the rate of introduction of the sample into the flame by a given sprayer. It was a study of these critical variables which led to the design of the sprayer used in the present instrument, with its high-suction, orifice-limited flow and dry spray.

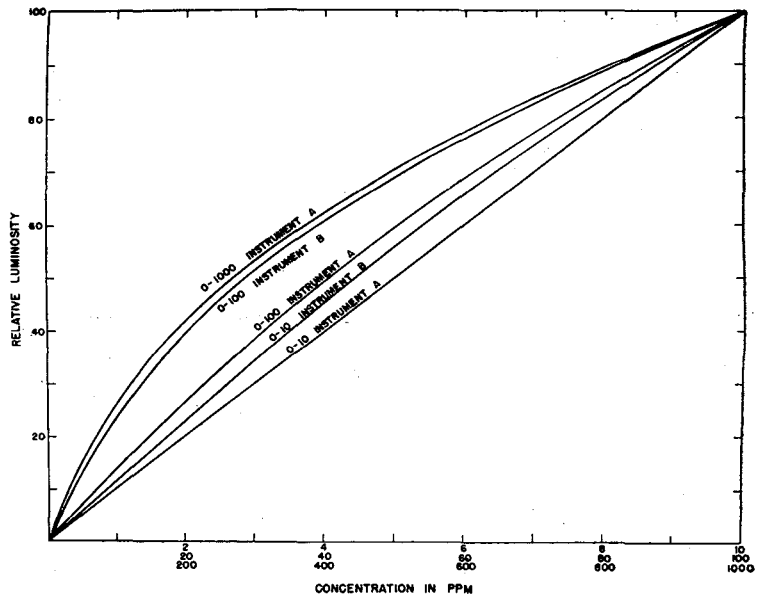

Figure 7. Calibration Curves for Sodium at 589 Mμ

Curves for range 0–1000, 0–100, and 0–10 p.p.m. are drawn between same initial and terminal points to show improvement in linearity at low concentrations. Two sets are given: for an earlier instrument, *A*, with unheated spray chamber and with propyl alcohol added to the Na solutions; and for a later and somewhat more sensitive instrument, *B*, with heated spray chamber, using purely aqueous solutions. Curves for *B* are applicable to determinations shown in Table I, A, C, and D

STANDARDIZATION

Internal versus External Standardization. The instrument may be standardized with either internal or external standards. The advantages of the latter, discussed below, led to efforts to design an instrument capable of convenient and accurate use with external standards.

As an internal standard a suitable element normally absent from the sample, such as lithium, is added in definite amount to the sample solution. Comparison of the relative emissions of the internal standard and of the element being determined gives a measure of the concentration of the latter. Internal standards tend to compensate for variations in viscosity, surface tension, and perhaps other variables. They have the disadvantage, however, that unsuspected errors may arise from their use. If the test sample should actually contain some of the internal reference element, a corresponding error would result. More serious is the fact that, contrary to the implications of some reported investigations, the emissions of the internal standard element and of the element being measured are usually influenced differently by variations in flame temperature and often by the presence of other components in the sample as well. The use of a foreign element for reference consequently may be misleading. Internal standards, therefore, while often useful, should be employed only after careful tests to determine the relevant facts.

In a versatile instrument the inconvenience of changing wave lengths in going from the elements being determined to the internal standard is an important disadvantage of the internal standard technique.

The preferable way of standardizing a flame spectrophotometer ordinarily is to prepare standard solutions which approximate the test samples in composition, and to standardize the instrument with the element that is being determined. In many cases a standard solution containing only the element being determined suffices. In other cases, particularly where interferences are encountered, it may be desirable to use standard solutions containing all the important constituents of the unknown in approximately the correct concentrations (cf. Table I, E and F). Disturbing effects often can be eliminated by working at sufficiently high dilutions. Where this step is insufficient or undesirable, self-compensating standards should be prepared. It often suffices to make a preliminary flame analysis of the unknown and then add to the standard solutions appropriate concentrations of other elements shown in the preliminary analysis. This often involves less effort than preparing an internal standard, particularly in routine determinations, and has the advantage that compensation for disturbing factors usually is much more complete than with an internal standard. As mentioned under Interference Effects, the comparison of diluted and undiluted samples against appropriate standards is an excellent precaution when sample composition is unknown or variable, and usually allows arithmetical correction for interferences of unknown origin.

Flame Background. The flame background must be subtracted from all readings. With many metals, such as the alkalies, the background value may be entirely negligible. In other cases it may be sufficiently small to permit use of a rough average value for a series of measurements. Wherever the background represents an appreciable fraction of the reading, as with such feebly luminous metals as tin and lead, the background value preferably should be obtained by measurement with pure solvent or, better, with a blank solution prepared in the same manner as the sample (cf. Table I, D, F, and G).

Linearity. It is characteristic of flame spectra that the curve of brightness versus concentration usually is substantially linear for low concentrations, with departures from linearity at higher concentrations. Typical calibration curves are shown in Figures 7, 8, and 9. Figure 7 shows the relative photocurrents for sodium in different ranges of concentration. The curves presented are the actual working curves employed in sodium determinations such as those of Table I. In practice, a family of curves is used, covering the rather small range of fluctuating conditions which would prevent one single curve from being applicable at all times. For purposes of mathematical interpolation, an equation can often be accurately fitted to the working curve. For sodium, the region from 50 to 250 p.p.m. accurately fits an equation of the form $c = A + Br^2$, where c is the concentration of

sodium and r is the photometer reading, A and B being constants whose ratio is nearly fixed for a given atomizer and flame adjustment but whose absolute magnitudes depend on slit width, etc. Between 0 and 100 p.p.m., an equation of the form $r = Ac - Bc^2$ was found to fit the curve very accurately. In the case of lithium (not shown), an equation of the form $c = r/(A + Br + Cr^2)$ was found to fit exactly from 0 to 250 p.p.m.

Figure 8 shows data for potassium, including the effect of flame composition (as defined by oxygen pressure for a given gas pressure) upon the curvature of the graph. This effect is especially pronounced in the case of potassium; with sodium it is observable but considerably smaller—i.e., a set of sodium calibration curves drawn between the same initial and terminal points for various flame compositions nearly coincide. The unusual S-curvature of the potassium curve is due to the fact that the peak luminosity of the potassium emission is found at lower oxygen pressures for smaller concentrations of potassium. Thus, with a particular burner, at 100 p.p.m. potassium the maximum intensity was obtained at about 5 or 6 inches of oxygen; at 200 p.p.m., at 7 inches; at 300 p.p.m., at 9 inches; and at 500 p.p.m., at about 10 inches. In other words, the smaller the potassium content of a flame, the cooler it must be to produce maximum excitation. With sodium

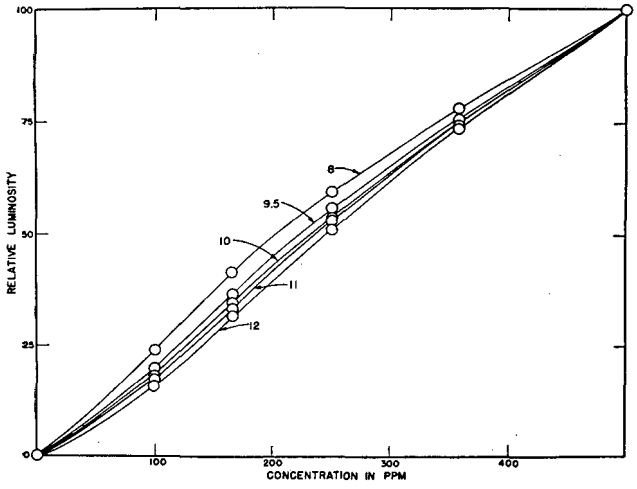

Figure 8. Calibration Curves for Potassium at 769 Mμ

Drawn between same initial and terminal points to reveal differences of shape. Gas pressure constant; oxygen pressure in inches of water shown as parameters

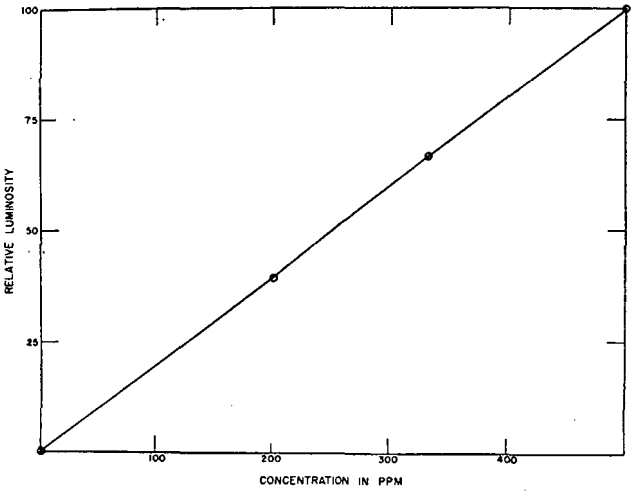

Figure 9. Calibration Curve for Calcium at 554 Mμ

the effect is of the same sign but much smaller: the optimal oxygen pressure shifts from 9.5 inches at 5 p.p.m. to 12 inches at 1000 p.p.m. It is interesting to note that, over a thousandfold range, the graph of optimal oxygen pressure versus the logarithm of sodium concentration would be nearly linear. In the light of this phenomenon, it may be understood that if for each concentration of potassium the corresponding optimal oxygen pressure were employed, the calibration curve would not only lose its inflection but would become nearly straight. Even without readjustment of the oxygen, in practical analyses linear interpolation may be resorted to without serious error over moderate ranges.

Figure 9 shows the strikingly linear relation obtained with calcium at its oxide band peak in the green. This circumstance permits highly accurate determinations to be made at all practical concentrations by simple comparison against a single standard, in the absence of interferences. Such graphs obtained at different emission wave lengths of the same metal may differ in curvature. Whereas the curve at 427 mμ for calcium has not been studied by the authors, in the case of sodium they have found that the weak emission at 330 mμ gives a practically straight graph up to several hundred parts per million. This wave length is therefore sometimes valuable for a preliminary analysis of a sodium solution, although because of the feebleness of the line, it is not suitable for precise determinations.

For most purposes the intercomparison technique for which the instrument is peculiarly suited yields sufficiently accurate results without the aid of a calibration curve. The latter is employed to show the permissible spread between sample and standard, and may be invoked if the desired precision warrants it. Sodium is an exception in that, as shown in Table I, A and C, a curve such as that of Figure 7 is almost always required except at the lowest concentrations.

SENSITIVITY FOR VARIOUS ELEMENTS

Time has not permitted an exhaustive study of the potentialities of the instrument, but at least preliminary information is available on 43 elements, listed in Table II. To the right of the name of each element is given the detection sensitivity, which is the least concentration at which the element can be detected easily by comparison against a suitable blank. In general, it represents the least concentration required to give a reading of 0.5% of full scale and equal to a galvanometer needle response of 1.5 scale divisions, under suitable adjustments of the various controls. It is hardly possible to generalize about the latter because of the widely varying conditions encountered. In the absence of serious interferences, a decidedly smaller concentration than that listed can usually be detected, and even in the presence of interferences, careful technique may yield measurements accurate to a fraction of the detection sensitivity. The attainable limit depends, however, upon the varying sensitivities of individual atomizers and spectrophotometers.

Wave lengths of flame emission are given in millimicrons. Opposite each wave length is given its intensity, in units obtained by dividing into 100 the detection sensitivity in parts per million for that particular wave length. The asterisk signifies an oxide band which may or may not cover a broad range of wave lengths but has its maximum at the wave length noted.

All intensity values are only approximate, because of the variability of external conditions, properties of the solution, and instrumental adjustments. Most of the intensities have been measured at National Technical Laboratories, using an oxygen–natural gas flame, but some of the data (for cadmium, gadolinium, gallium, gold, indium, mercury, palladium, rhodium, rubidium, ruthenium, silver, strontium, thallium, and yttrium) have been taken or recalculated from the work of Lundegårdh and others, using other kinds of flame, and have not been verified by the authors.

Table II. Flame Spectra of Elements

Aluminum

No emission

Antimony

No emission

Barium, 1 p.p.m.

490–570, including:	
520*	20
550*	20
745*	100
830*	50
873*	50

Boron, 5 p.p.m.

345	0.3
454*	2
473*	5
495*	10
521*	15
548*	20

Cadmium, 500 p.p.m.

326.1	0.2

Calcium, 0.3 p.p.m.

422.7	100
554*	200
603*	100
624*	300
648*	100

Cesium, 0.1 p.p.m.

455.5	1
459.3	0.5
621.3	
672.3	
852.1	1000
894.4	

Chromium, 3 p.p.m.

357.9	30
359.3	30
360.5	30
425.4	20
427.5	20
429.0	20
520.6	10
527*	10

Chromium (*Contd.*)

539*	10
544*	10
559*	10
564*	10
582*	10
609*	20
645*	30
685*	20
850*	3

Cobalt, 5 p.p.m.

238.9	
304.4	1
350.2	20
352.7	20
412.1	1

Copper, 1 p.p.m.

324.8	100
327.4	90
512*	5

Dysprosium

Determinable

Europium

Determinable

Gadolinium

451.4*	
461.4*	
569.6	

Gallium, 1 p.p.m.

403.3	
417.2	100

Gold, 50 p.p.m.

267.6	2
365.2*	
397.5*	0.1

Indium, 1 p.p.m.

303.9	
325.6	
410.2	
451.1	100

Iron, 10 p.p.m.

344.1	2
372.0	7
373.6	10
386.0	7

Lanthanum

438.4*	
443.3*	
544*	
563*	
714*	
745*	
798*	
860*	

Lead, 300 p.p.m.

283.3	0.1
364.0	0.1
368.3	0.2
405.8	0.3

Lithium, 0.05 p.p.m.

460.3	0.1
670.8	2000

Magnesium, 10 p.p.m.

285.2	10
370.8	10
382.9, 383.2	8

Manganese, 1 p.p.m.

279.8	1
403.4	100
510*	10
541*	30
561*	70

Mercury, 50 p.p.m.

253.6	2

Neodymium

Determinable

Nickel, 3 p.p.m.

299.4	1
300.3	5

Nickel (*Contd.*)

303.8	2
324.3	1
331.6	2
337.0	7
338.1	5
339.3	10
341.5	15
342.4	4
343.4, 343.7	10
344.6	10
345.8, 346.2	20
347.3	5
348.4	2
349.3	15
350.1	3
351.0, 351.5	20
352.4	30
354.9	1
356.6, 357.2	10
359.8	4
361.0	8
361.9	15
367.4	1
373.7	1
377.6	3
378.4	3
380.7	3
385.8	6
515*	3

Palladium, 50 p.p.m.

340.5	2
363.5	2

Phosphorus

No emission

Platinum

Determinable

Potassium, 0.05 p.p.m.

344.6	0.5
404.4, 404.7	7
766.5, 769.9	2000

Praseodymium

Several good bands

Rhodium

332.3	
339.7	
343.5	
352.8	
358.3	
359.6	
369.1	
369.6	
449.2	

Rubidium, 0.1 p.p.m.

420.2	3
421.6	1
780.0	1000
794.8	

Ruthenium, 30 p.p.m.

372.7	3
378.6	3

Samarium

Determinable

Scandium

Determinable

Selenium, 5000 p.p.m.

359*	0.02

Silver, 2 p.p.m.

328.1	7
338.3	50
520.9	

Sodium, 0.01 p.p.m.

330.2	7
589.0, 589.6	10,000

Strontium, 0.5 p.p.m.

407.8	10
421.6	10
460.7	200
870*	

Thallium, 1 p.p.m.

276.8	0.1
351.9	2
377.6	80
535.0	10

Tin, 500 p.p.m.

320–370, including:	
326.2	0.2
332*	0.2
348*	
358*	
452.5	0.1

Yttrium

464.4	
467.6	
486.0	

Zinc

No emission

SUMMARY

It is believed that the Beckman flame spectrophotometer offers a combination of features not hitherto available. Of particular significance are:

Available accuracy of a few tenths of 1% of the amount present. Emission measurements equal in analytical accuracy to absorption measurements.

High resolving power, affording relative freedom from optical interference effects.

High sensitivity which permits analysis to be made at trace concentrations, or at high dilution to minimize emission interferences.

Speed and convenience. No cleaning between samples. Several readings per minute.

Small samples. Only a single drop of solution is consumed per reading.

Hot flame. Excites more than half the elements.

Low initial and operating cost in comparison with other high resolution emission equipment.

It is believed that these features will greatly extend the field of utility for flame spectrophotometry. On the one hand, the improved quantitative accuracy permits flame spectrophotometry to be used for many analytical determinations where more time-consuming volumetric or gravimetric methods have heretofore been required. On the other hand, the extension of flame technique to embrace additional elements enables the analyst to replace arc spectrography with the more accurate, more convenient, and less expensive flame spectrophotometry.

ACKNOWLEDGMENT

Thanks are due G. L. Locher, who worked out the design of the burner and performed preliminary experiments on reflux type atomizers.

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A PHOTOELECTRIC TITRIMETER

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A photoelectric titration apparatus (titrimeter) has been designed employing two barrier-layer cells and narrow-band glass filters in a current bridge circuit. The theory of the measuring circuit has been derived and several methods of operation of the apparatus are suggested.

IN VOLUMETRIC analysis any property of the solution which exhibits a definite change at the equivalence point may be used for its recognition. Generally, preference is given to indicator methods where a change in the color, fluorescence, or turbidity can be detected visually, without special apparatus. However, when no suitable indicator is available or when the change is too vague, the measurement of a physicochemical property is used. Still, the lack of precision, which is usually associated with the visual detection of indistinct color or turbidity changes, can often be overcome by the utilization of a photoelectric titration.

The first attempt to overcome the uncertainty of the visual detection of color changes was made in 1918 by Tingle (*10*). A summary of the previous work and a significant contribution to the design of a photoelectric titration apparatus were made by Osborn and his co-workers (*9*) in 1943. Their instrument employed two barrier-layer photocells connected with two variable resistors and a galvanometer in a Wheatstone bridge circuit originally used by Wilcox (*12*) in a colorimeter. The high differential sensitivity afforded by the two-cell method was assured by the use of a colored glass filter in front of each photocell. The filters were chosen so that one had a maximum transmittance in the spectral region of one absorption band of the selected indicator, while the other had its maximum transmittance in the region of another band or else in the region where little or no change in the absorption took place.

Further contributions have been made by Weber (*11*), Havemann (*4*), Chepelevetskiĭ (*2*), Luré and Tal (*6*), Garcia Escolar (*3*), and Lambert (*5*).

In a previous investigation (*8*) of the titration of fluorine with cerous nitrate using methyl red, sometimes the visual detection of the equivalence point was very difficult. In an attempt to improve this, the authors devised the present instrument for a photoelectric method.

DESCRIPTION OF INSTRUMENT

The apparatus, constructed of aluminum, is shown in Figures 1 and 2. Light from a 100-watt projection lamp collimated by a lens enters the sample compartment through a rectangular window. The parallel beam then passes through a glass absorption cell containing the solution to be titrated and emerges through two circular, adjacent windows. In this manner the original beam is split into two parts, one of which passes in a straight line to one barrier-layer photocell while the other is reflected by a mirror through 90° and strikes the other photocell. Instead of this arrangement, a single beam and a dividing prism may be used. The two photocells are balanced to zero current against one another, either electrically by a slide-wire bridge arrangement or optically by changing the light on either cell with the iris diaphragms in front of each photocell. Essentially monochromatic light is realized in each beam by colored glass filters in the compartments in front of each diaphragm.

The lamp socket is mounted on an adjustable platform attached to a tripod base. The lamp is cooled by air drawn in through a chimney directly above the lamp on the top panel and out through a screened window in a side panel by a fan rotated by a small induction motor. The fan and motor assembly was obtained as a unit through the courtesy of the Photovolt Corporation. Stirring is accomplished by means of a magnetic stirrer mounted under the sample compartment. Both the intensity of the lamp and the speed of the stirrer can be regulated by individual rheostats mounted on the top panel. The 110-volt alternating current line furnishes the power supply for the titrimeter lamp, fan motor, stirrer motor, and step-down transformer for the galvanometer lamp.

Two glass absorption cells with cemented plane-parallel walls have been used, with light paths of 56 and 32 mm.

Access to the sample and filter compartments is gained through cutouts in the top panel, each of which is provided with a cover. Originally, in order to exclude stray light, the cover to the sample compartment had been provided with a hole and short tube for admitting the buret tip; however, experience has shown that the use of the cover for this purpose during a titration is unnecessary. Nevertheless, the arrangement becomes useful for excluding carbon dioxide during neutralization reactions. The filter compartments are designed to accommodate any one of the set of 2-inch (5-cm.) square monochromatic filters which are accessory equipment for the Lumetron colorimeter Model 402-E. Other special features include a drain hole in the sample compartment, dials attached to the rotating parts of the iris diaphragms and projecting out of the top panel, and frosted glass plates in front of the photocells.

The photocells are photovolt Model 735 barrier-layer cells with Lucite mountings and hermetically sealed. They are connected with two rheostats and a galvanometer in the balanced bridge circuit shown in Figure 3. It is a current bridge rather than a voltage bridge. Wood (*13*) has shown that this arrangement of

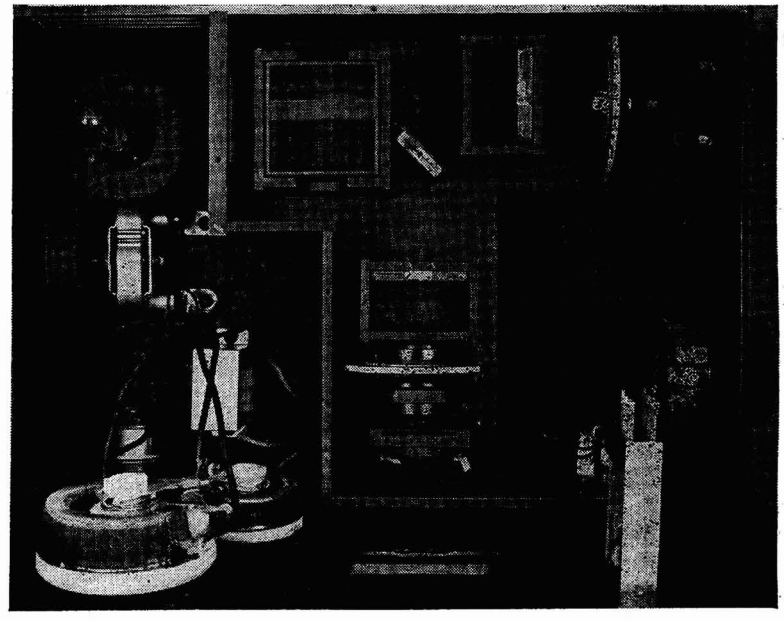

Figure 1. Photoelectric Titration Apparatus

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having the photocells connected so that the terminals of opposite polarity are joined, with the galvanometer in parallel with these cells, has advantages with respect to sensitivity, linearity of response, and stability. The rheostats are of the General Radio Company Type 214-A (500 ohms), each equipped with that company's Type 703-B friction-drive dial. The graduations on the dials were extended to cover the full arc of the rheostat. The galvanometer is a General Electric Model 32-C242G9 multiple-reflection galvanometer with a sensitivity of about 0.0057 microampere per division. Not shown in the circuit diagram is a 2PDT anticapacity switch which, in one position, connects both photocells in the circuit and, in another, only the direct photocell.

ANALYSIS OF THE CIRCUIT

Wood (*13*), Brice (*1*), and Müller (*7*) have presented analyses of this and related circuits. Müller derived an expression for the transmittancy of a solution interposed in only one of the light paths for the case in which no optical filters are employed. With

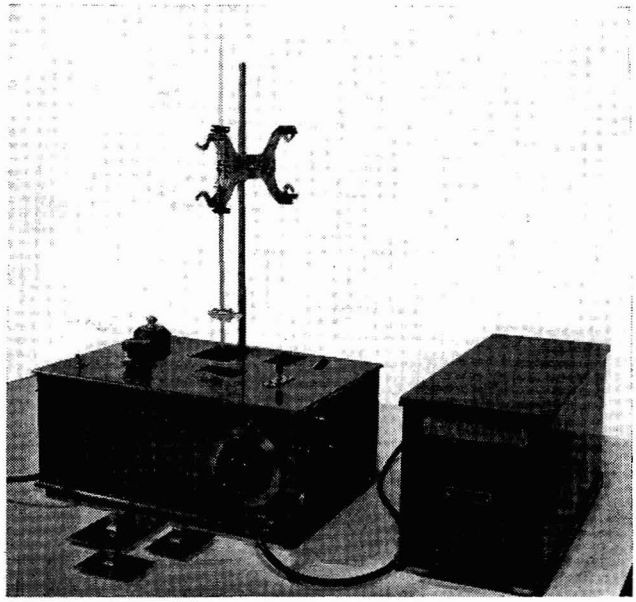

Figure 2. Photoelectric Titration Apparatus

this condition, which is found in an ordinary photometer, good compensation for variations in the intensity of the light source is effected. This is true not only because of the balanced circuit, as Müller has shown, but particularly when a monochromatic filter is used, for then differences in the spectral response of the photocells within the narrow spectral range isolated by the filter cannot be of any consequence. However, in an instrument of the sort herein described, in which different filters are interposed in the light paths, compensation for fluctuations in the intensity of the light source might be unsatisfactory if the spectral response of the photocells, or the transmittances of the filters, were markedly different at the two selected wave bands. This difficulty could easily be corrected by the utilization of a voltage regulator attached to the alternating current line.

The circuit, shown in Figure 3, gives the following analysis:

In one arm of the circuit the current, i, generated by the photocell is divided into two parts, i_1 and i_2, in the inverse ratio of the resistances of the parallel circuits as determined by the position of the slide-wire.

$$i = i_1 + i_2$$

$$i_1 = i\left[\frac{(1 - x)R + r_g}{R + r_g}\right]$$

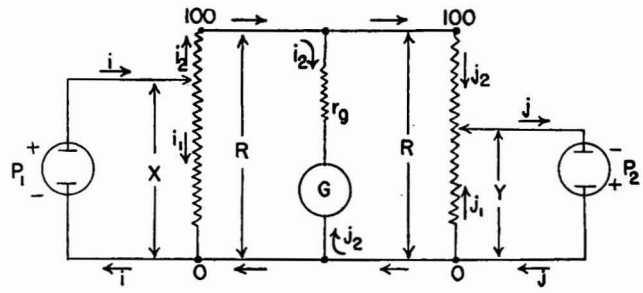

Figure 3. Circuit Diagram

P_1, P_2. Photocells
G. Galvanometer
R. Resistance of rheostats
r_g. Resistance of galvanometer

$$i_2 = i\left[\frac{R \times x}{R + r_g}\right]$$

where

R = resistance of the rheostat
x = dial reading/100
r_g = resistance of the galvanometer

Similarly, for the other arm of the circuit

$$j = j_1 + j_2$$

$$j_1 = j\left[\frac{(1 - y)R + r_g}{R + r_g}\right]$$

$$j_2 = j\left[\frac{R \times y}{R + r_g}\right]$$

This being a current bridge, the condition for balance is

$$i_2 = j_2$$

or,

$$i \times x = j \times y$$

i.e., the photocurrents are inversely proportional to the dial readings. Because for low light intensity and small external resistance the photocurrent flowing in a barrier-layer cell circuit is directly proportional to the light intensity striking it,

$$i = k_1 I_1 \qquad \text{(direct cell)}$$

and

$$j = \alpha k_2 I_2$$

where k_1 and k_2 are constants depending on the characteristics of the photocells and the spectral transmittances of the colored glass filters, and α is the reflectivity of the mirror.

Since

$$\frac{I^\lambda}{I_0^\lambda} = T^\lambda$$

where I^λ and I_0^λ are the transmitted and incident light intensities, respectively, and T^λ is the transmittancy of the solution at wave length λ, then

$$i = k_1 I_0^{\lambda 1} T^{\lambda 1}$$

and

$$j = \alpha k_2 I_0^{\lambda 2} T^{\lambda 2}$$

so that the condition for balance becomes

$$k_1 I_0^{\lambda 1} T^{\lambda 1} \times x = \alpha k_2 I_0^{\lambda 2} T^{\lambda 2} \times y$$

or

$$\frac{x}{y} = \frac{\alpha k_2 I_0^{\lambda 2}}{k_1 I_0^{\lambda 1}} \times \frac{T^{\lambda 2}}{T^{\lambda 1}}$$

For a fixed light intensity and a given set of photocells and filters this expression is simply

$$\frac{x}{y} = k \times \frac{T^{\lambda 2}}{T^{\lambda 1}}$$

Consequently, for this type of balanced circuit and the arrangement in which an optical filter of one color is used with one photocell while a filter of a different color is used with the other photocell, the rheostat dial readings are inversely proportional to the transmittancies of the solution at the two selected wave bands.

METHOD OF OPERATION

One method of operation is that used by Osborn and coworkers. The end point was defined by the intersection of two straight lines extrapolated, as in a conductometric titration, through points obtained by plotting the logarithm of the varied resistance values against the volume of titrant. The authors made no effort to explain their choice of functions for plotting, but because of the similarity in the expressions for the two balanced circuits in point, it seems appropriate to do so here in a discussion which is intended to apply to both circuits. For the sake of simplicity in the comparison the two expressions will be reduced to this common one, $x/y = kT_2/T_1$, in which T_1 and T_2 are equivalent to $T^{\lambda 1}$ and $T^{\lambda 2}$, respectively, while x and y will be retained and are equivalent to R_1 and R_2, respectively.

This gives four possibilities of titration. In the first two cases, if the color filters are selected so that one transmits light in a region of the spectrum where the transmittancy of the solution with indicator does not change appreciably upon the addition of titrant, T_1, while the other transmits in that region where the transmittancy of the solution changes most markedly, T_2, then balance can be restored by varying (1) x as y remains fixed, or (2) y as x remains constant.

Case I. T_1 and y fixed, x varies directly with T_2.

$$\frac{x}{y} = k \frac{T_2}{T_1}$$

$$x = \frac{ky}{T_1} \times T_2$$

Since

$$T_2 = 10^{-\mu_2 c_2}$$

where

μ_2 = molar extinction coefficient
c_2 = molar concentration of one of the two colored forms of the indicator

$$x = \frac{ky}{T_1} \times 10^{-\mu_2 c_2}$$

$$\log_{10} x = \log_{10} \frac{ky}{T_1} - \mu_2 c_2$$

or

$$\log_{10} x = \phi_1 - \mu_2 c_2$$

(equation for a straight line)

Case II. T_1 and x fixed, y varies inversely with T_2.

$$\frac{x}{y} = k \frac{T_2}{T_1}$$

$$y = \frac{T_1 x}{k} \times \frac{1}{T_2}$$

$$y = \frac{T_1 x}{k} \times 10^{-\mu_2 c_2}$$

$$\log_{10} y = \log_{10} \frac{T_1 x}{k} \times \mu_2 c_2$$

$$\log_{10} y = \phi_{II} + \mu_2 c_2$$

Except for dilution effects, one would expect no change in the spectrum of the solution with the addition of titrant until the color of the indicator begins to change, so that, when the logarithm of the dial readings is plotted against the volume of reagent added, one should obtain a straight line nearly horizontal to the abscissa. After the end point has been reached, if the color of one of the forms of the indicator changes in intensity in accordance with the Lambert-Beer law with the addition of titrant, the same plot will give a straight line, as indicated by the previous derivation, which is nearly vertical. The slope of this line will depend upon which rheostat is varied; but, in order to make it positive, Case I must be applied when the primary absorption band (and therefore c_2) is decreasing at the end point, and Case II when it is increasing.

In the actual operation of the present instrument the rheostat to be moved is set at some low value and balanced by the adjustment of the other rheostat, which thereafter remains fixed throughout the titration. After the end point has been reached, the intensity of the variable beam and therefore the current of its photocell will either rise or fall, necessitating the adjustment of the variable rheostat to a higher reading for balance in both Cases I and II, if the proper selection has been made.

The other two cases occur with phthalein indicators which pass through their color zones with a decrease in an absorption band in one portion of the spectrum and an increase in a second band in another. If the filters are chosen to transmit in these spectral regions, sensitivity is considerably increased.

Case III. y fixed, x varies directly with T_2, and inversely with T_1.

$$\frac{x}{y} = k \frac{T_2}{T_1}$$

$$x = ky \times \frac{T_2}{T_1}$$

$$x = ky \times \frac{10^{-\mu_2 c_2}}{10^{-\mu_1 c_1}}$$

$$\log_{10} x = \log_{10} ky - \mu_2 c_2 + \mu_1 c_1$$

but

$$c = c_1 + c_2$$

Therefore,

$$\log_{10} x = \log_{10} ky + \mu_1(c - c_2) - \mu_2 c_2$$

$$\log_{10} x = \log_{10} ky + \mu_1 c - c_2(\mu_1 + \mu_2)$$

$$\log_{10} x = \phi_{III} - (\mu_1 + \mu_2)c_2$$

Case IV. x fixed, y varies directly with T_1 and inversely with T_2.

$$y = \frac{x}{k} \times \frac{T_1}{T_2}$$

Because the derivation is very similar, only the final expression is given here.

$$\log_{10} y = \phi_{IV} + (\mu_1 + \mu_2)c_2$$

Obviously, the effect of making this choice of filters is to in-

crease the value of the slope of the nearly vertical line. Consequently, the oblique angle which this line makes with the horizontal line approaches 90° more closely than it does for the other choice of filters, so that the point of intersection of the two lines is more sharply defined.

A large indicator concentration and small increments of titrant are necessary in order to get a sufficient number of points for the vertical line. Unless the color change is gradual enough, the operator may fail to detect the vertical portion of the curve even with very small increments.

A second and more rapid method for defining the end point follows directly from the expression which relates the rheostat dial readings to the transmittancies of the solution at the two selected wave bands. With the usual indicator concentrations the change in the optical density—i.e., absorbency—of an indicator, at the wave band for which this change is greatest, passes through a fairly sharp maximum upon the addition of titrant in the vicinity of the end point. In other words, in this instance the vertical portion of the curve of the logarithm of the dial reading versus the volume of titrant exhibits an inflection point rather than a straight-line portion. The inflection point represents the point of maximum color change of the indicator.

If the absorbency ($-\log_{10} T$, where T is the transmittancy) passes through a maximum change, one can expect the transmittancy itself to do likewise, although not for exactly the same volume of titrant. The difference in these volumes is smaller the sharper the color change, and a standardization by the same method or a blank would correct for any error.

Consequently, for Case I, for example, because x is directly proportional to T_2, with the above assumption it becomes necessary only to observe the maximum change in the rheostat dial readings for several additions of titrant in the vicinity of the end point. For Case II the maximum change in the reciprocal of the dial readings corresponds to the end point. Cases III and IV are similar, except that here the change in the ratio of the transmittancies at the selected wave bands passes through a maximum. This is evident, for, if T_1 goes through a smaller change but in the opposite direction as T_2 at the same time, obviously its effect will be to magnify the change in T_2.

If the maximum change in the transmittancy occurs at a point too far removed from the maximum change in the absorbency for the accuracy desired, the maximum change in the logarithm of the dial readings may be observed in all four cases. This may yield more accurate results because the logarithm of the dial reading may be at least approximately a linear function of the logarithm of the transmittancy, or the absorbency.

Another method for operating the titrimeter is especially suitable for rapid, routine analyses. Initially, with a standard reference solution in the sample compartment, the circuit simply is balanced to zero current either optically with the iris diaphragms or electrically with the rheostats. Then the reference solution is replaced with an unknown solution which is titrated until the galvanometer reaches the balanced position. The selection of the color filters is made on the same basis as before. The accuracy of the method depends on the judicious selection of an indicator and the reliability of the standard, while the precision, which is usually excellent, depends on the reproducibility of experimental conditions, chiefly the indicator concentration.

For turbidity titrations with an instrument of this sort only a single photocell can be used because, with two cells, an increase in the turbidity would reduce the intensity of both beams. Actually, turbidity titrations can be performed well with an ordinary two-cell photometer for which the sample is interposed in only one of the light beams. For a single-cell instrument one usually reads the deflections of a galvanometer, but unless a voltage stabilizer is used there may be considerable uncertainty attached to these readings. In order to obviate this difficulty for the titrimeter the galvanometer spotlight was returned by means of the rheostat to within 10 divisions of the null position after each addition of titrant, and the dial reading was observed. The expression relating the deflection, *d*, to the dial reading, *r*, is:

$$r \times d = 1000$$

In this manner a dial reading of 10 corresponds to a galvanometer deflection of 100, a dial reading of 50 to a deflection of 20, etc. For titrations to maximum turbidity the maximum dial reading corresponds to the end point. Unfortunately, the sensitivity and reproducibility of the adjustment of the rheostat diminish as the deflection decreases, so that they are least at maximum turbidity. Titrations to the appearance or disappearance of a precipitate do not present this problem.

SUMMARY

A new differential photoelectric titration apparatus employing two barrier-layer cells in a current bridge circuit has been designed, into which several improvements over the previous instruments have been incorporated.

The apparatus has the advantage of a high differential sensitivity, and in this respect is superior to other two-cell and all one-cell methods. There are two disadvantages of very minor importance: Only a change in the intensity rather than an absolute value can be measured, and there is some loss in compensation for line voltage fluctuations. The first is not important because in titrations one is interested only in the change in light intensity, and if absolute measurements are desired reference standards could be used. The second disadvantage can be corrected by the use of a voltage stabilizer.

Among the improvements in design which have been made are the use of narrow-band filters, a magnetic stirrer, iris diaphragms for optical compensation, and a bridge circuit which is superior to other circuits in stability and linearity of response.

On the other hand, several changes or additions which could be made to the original design have become apparent. In the first place, it would be better to divide the light beam by means of a partially reflecting mirror or dividing prism in order to be able to accommodate titration cells of various sizes. As a further precaution against heat effects from the lamp, the interior panels around the lamp might be lined with asbestos or the lamp might be placed in a separate housing. However, the latter would lose the advantage of a single-unit apparatus.

The theory of the measuring circuit has been derived and several methods of operation of the apparatus have been suggested.

Applications of this instrument to various titrations will be given in a subsequent paper.

ACKNOWLEDGMENT

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Titrations with a Photoelectric Titrimeter

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Various neutralizations and a precipitation titration have been made with a photoelectric titrimeter, which has also been applied to the titration of fluorides and to the determination of the critical concentration of soap solutions.

THE authors (*17*) have described a photoelectric titrimeter and its principle of operation. Müller (*16*) has shown that compensation for variations in the intensity of the light source is inherent in the circuit used. This instrument has been applied to several titrations, some of which are very difficult by visual methods, to show its precision and stability.

NEUTRALIZATION REACTIONS

The spectrophotometric study by Brode (*3*) shows that an ideal hydrogen ion concentration indicator has two narrow, sharp absorption bands both in the visible and yet separated far enough so that one does not affect the height of the other. When one absorption maximum decreases the other should increase, and there should be a relatively large change in the height of these maxima in relation to the change in hydrogen ion concentration. Holmes (*11*, *12*) has shown that the indophenols satisfy these conditions, but that some are relatively unstable in aqueous solution. Except for the fact that their secondary bands are in the violet region, the phthaleins and sulfonphthalein are satisfactory. The azo dyes have broad bands which affect each other's height and show a definite shift (*9*) toward the violet with increased pH.

Holmes (*11*) was the first to point out that the maximum degree of alteration per unit change in indicator transformation is obtained by measuring the absorbencies at the two wave lengths at or near the maxima of the two absorption bands. Furthermore, the ratio of the absorbencies of the two bands does not vary with indicator concentration, and dichromatism introduces no interference.

For all neutralization titrations the method of balancing the circuit with a buffer solution and then titrating to this balanced position was used. The precision of this method depends upon the ability to reproduce a given difference in intensities, which necessitates a careful control of the indicator concentration. However, with fairly high indicator concentrations, with the ratio of the intensities of the two beams near unity, and with a pH near the middle of the indicator range the error is slight. It may be preferable to use as a second filter one which transmits in the region where little or no change in the absorbency of the indicator occurs rather than one which transmits in the violet region, as in the latter case one may have to compensate for large differences in the transmittances of the filters as well as in the responses of the photocells.

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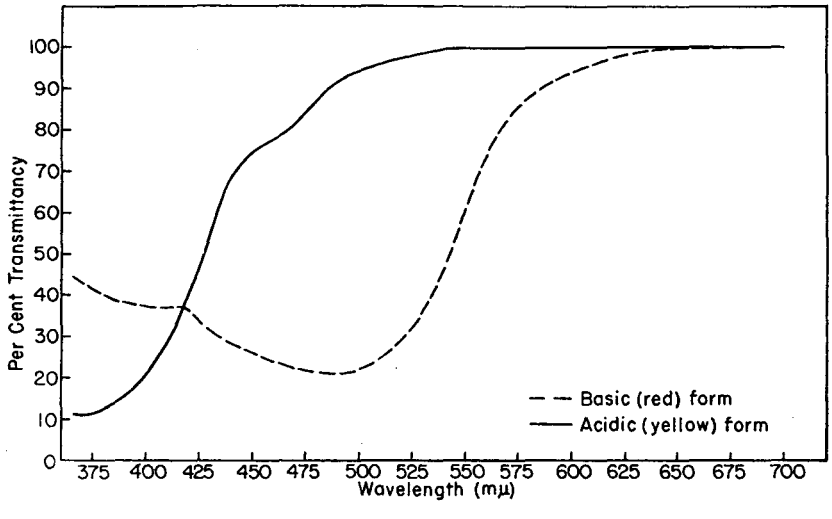

Figure 1. Transmittancy Curves for Alizarin Yellow R

Sodium Hydroxide–Hydrochloric Acid. In these titrations 20 drops of a 0.1% isohydric solution of bromothymol blue in a final volume of 150 ml., filters with transmittance-band maxima at 420 and 620 mμ (*3*), and a Clark and Lubs buffer solution of a pH of 6.75 were used. Titrations were made to determine the dilution limit with strong acids and bases. The solutions and water were boiled to remove carbon dioxide and the titration was completed in an atmosphere of nitrogen. The following averages of several titrations were obtained:

Normality	NaOH, Ml.	HCl, Ml.
0.1	24.33	25.00
0.01	24.33	25.00
0.002	24.37	25.00

Acetic Acid–Ammonium Hydroxide. As a further indication of the precision, these two solutions were titrated as above and the average values were compared with the average obtained visually by standardizing the acetic acid against sodium hydroxide with phenolphthalein and the ammonium hydroxide against hydrochloric acid with modified methyl orange.

Visual			Titrimeter		
Normality, $HC_2H_3O_2$	Normality, NH_4OH	Ratio	$HC_2H_3O_2$, Ml.	NH_4OH, Ml.	Ratio
0.09476	0.10325	1.088	25.05	23.04	1.088
0.09493	0.10317		25.05	23.01	

Boric Acid–Sodium Hydroxide and Aniline–Hydrochloric Acid. The possibility of making a direct titration of very weak acids and bases was investigated with these solutions, using alizarin yellow R and methyl yellow as the best available indicators. However, these titrations were found to be impractical. The absorption spectra of alizarin yellow R (Figure 1) showed it to be unsatisfactory and the potentiometric titration of aniline (Figure 2) showed that a variation of 40% in the salt concentration would cause an error of about 2% in the equivalent volume of the titrant, assuming the pH at the equivalence point to be fixed. Therefore, the final volume and sample weight must be constant or volume corrections must be applied.

Titrations with Mixed Indicators. A differential instrument of this type should be particularly well adapted for certain mixed indicators. The spectral transmittance curves (*9*) of equal parts of methyl red and bromocresol green show that this mixed indicator has the advantage that it exhibits two absorption bands in the middle of the visible

portion of the spectrum, whose intensities change in opposite directions at the same time. Modified indicators, however, offer no improvement over the indicators themselves with an instrument of this type.

The mixed indicator of 3 parts of bromocresol green and 2 parts of methyl red exhibits a sharp change from green to wine-red at a pH of 5.1. Potentiometric titrations of 0.1 *N* and 0.01 *N* solutions of hydrazine and hydrochloric acid gave the equivalence points at pH values of 4.9 and 5.2, respectively. When 25.00 ml. of 0.1 *N* and 0.01 *N* hydrazine were titrated with hydrochloric acid, using filters of 515 and 620 mμ, the following values were obtained:

Normality	HCl, Ml.	N_2H_4, Ml.
0.1	27.15 27.16 27.16	25.00
0.01	27.15 27.16 27.14	25.00

Potentiometrically, the equivalent volume of hydrochloric acid was between 27.10 and 27.20 ml. in both cases.

A mixture of 3 parts of cresol red and 3 parts of thymol blue has no significant advantage over either with the titrimeter because the alkaline violet color is a single broad absorption band caused by the superposition of the thymol blue band at 596 mμ upon that of the cresol red at 572 mμ.

TURBIDITY TITRATIONS

The phototurbidimetric titration of barium sulfate has been made by del Campo and co-workers (*4*, *5*) and by Ringbom (*20*). The single photocell method of operation, in which the galvanometer spotlight is returned to ten divisions from the null position after each addition of titrant, was used.

Titration to maximum turbidity is based upon the principle that the addition of each portion of the precipitant results in the formation of some precipitate and an increase in the turbidity of the solution. An increase in the absorption of light takes place until the equivalence point is reached, and further addition of the precipitant decreases the degree of turbidity by dilution. If coagulation is prevented, the equivalence point corresponds to maximum turbidity.

Ringbom gave the optimum experimental conditions for the precipitation of barium sulfate as:

1. The concentration of the sample must be high enough for one drop of precipitant to cause immediate precipitation but low enough for the precipitate to remain in suspension. Fulfillment of these two conditions is aided by the addition of a miscible organic solvent and a protective colloid.
2. The concentration of precipitant should be such that one drop corresponds to the accuracy of measurement.
3. The thickness of layer should be such that the loss in illumination at the end point is 40 to 80%.

Ringbom's procedure was followed using a 0.02994 *M* solution of barium chloride and a 0.02979 *M* solution of sodium sulfate. A 490 mμ filter was used to decrease the level of illumination. The sulfate was added to the barium salt, although no detectable difference was noticed with the reverse procedure. The barium chloride solution was placed in a 90-ml. titration cell with a 32-mm. light path, 35 ml. of water, 25 ml. of 95% ethyl alcohol, and 1 ml. of a freshly prepared 1% solution of gum arabic were added, and the solution was stirred for a few minutes. About 5 ml. of the sulfate solution were added at a rate sufficient to cause the formation of very small particles and then the addition was continued in 0.10 ml. increments at first and in 0.05-ml. increments near the end point. After each addition the solution was stirred for 1 to 2 minutes and the galvanometer light was adjusted, the maximum dial reading corresponding to the equivalence point. The following values were obtained:

0.02994 *M* $BaCl_2$ Used, Ml.	Na_2SO_4 Observed, Mg.	Na_2SO_4 Calculated, Mg.	Deviation, Mg.
5.77	24.59	24.55	0.04
6.13	25.94	26.07	0.13
6.24	26.49	26.53	0.04

The presence of the alcohol causes immediate precipitation of barium sulfate and the gum arabic maintains a finely dispersed precipitate but, even so, *Schlieren* lines were sometimes observed with 0.02 *M* solutions and invariably with 0.01 *M* solutions. Occasionally they appeared at the start of the titration with 0.03 *M* solutions if the first 5 ml. were not added rapidly enough. The best results were obtained with the maximum turbidity at a dial reading between 20 and 35.

TITRATION OF FLUORIDE WITH THORIUM NITRATE

Originally this investigation was begun on the design (*16*) and application of an instrument to the titration of fluoride with cerous nitrate, using methyl red (*18*). However, because of the variation of the pH at the equivalence point and the difficulty of maintaining a constant 80° C. temperature, attention was directed to this titration.

Willard and Winter (*24*) introduced a procedure using a lake of zirconium and alizarin red as indicator in 50% alcohol. Armstrong (*2*) improved the method by omitting the use of zirconium and using the lake with thorium. Other workers have confirmed this procedure, although the pH must be controlled carefully. Hoskins and Ferris (*13*) state that the optimum pH is approximately 3.5 to 50% ethyl alcohol and recommend an equimolar solution of monochloroacetic acid and sodium monochloroacetate in an amount sufficient for a buffer concentration of 0.02 *M*. They used 0.04 ml. of a 0.05% aqueous indicator solution in 50 ml. and titrated to the very light pink shade of a matching blank. Later Armstrong (*1*) and Rowley and Churchill (*21*) both found the color change sharper in aqueous than in 50% alcoholic solution with a pH from 2.9 to 3.1. Reynolds and Hill (*19*) confirmed these indicator results and found that the concentration of indicator, the fluoride concentration, and the temperature also affected the titration. They also reported a small blank in aqueous solution which increased with the volume. Approximately the same

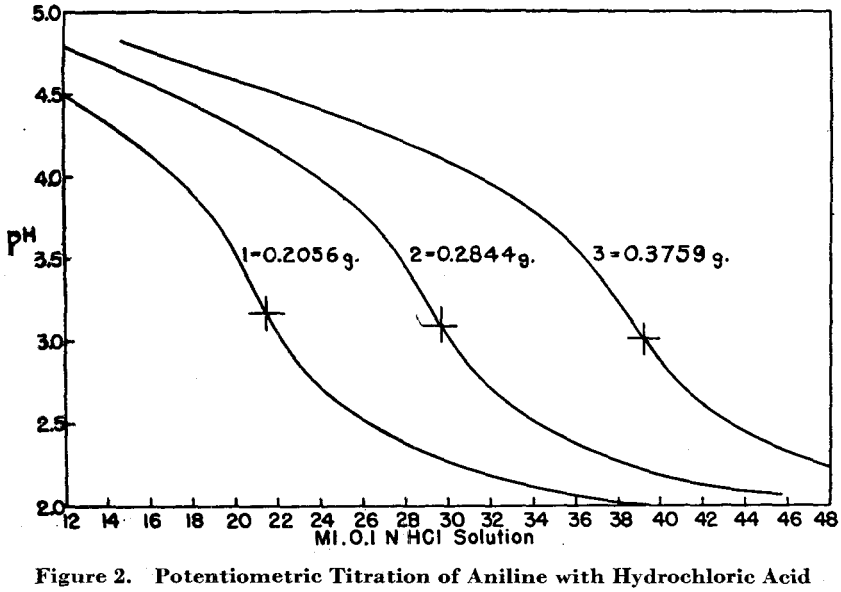

Figure 2. Potentiometric Titration of Aniline with Hydrochloric Acid

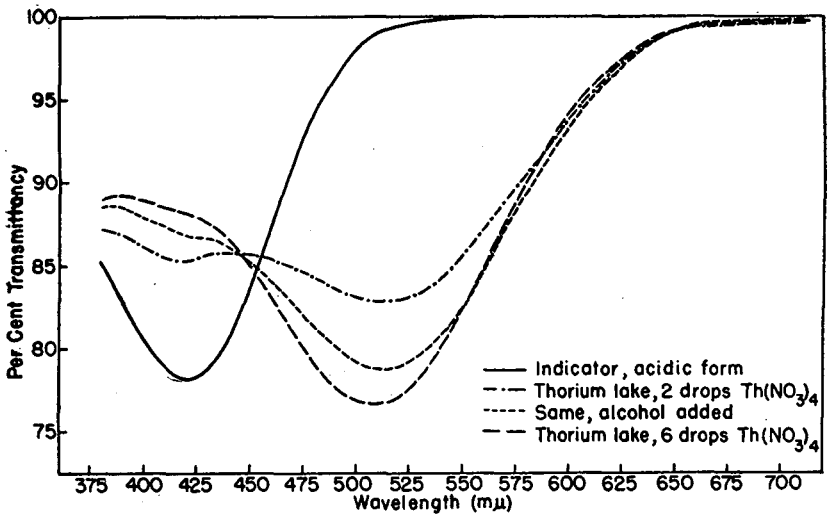

Figure 3. Transmittancy Curves of Sodium Alizarin Sulfonate and Thorium Lake

Table I. Titration of Fluoride

0.05 *N* $Th(NO_3)_4$, Ml.	1-Min. Interval Dial reading	Change	2-Min. Interval Dial reading	Change
	Aqueous Solutions			
4.40	99.2		98.8	
		2.0		2.8
4.50	97.2		96.0	
		3.9		5.3
4.60	93.3		90.7	
		6.9		8.6
4.70	86.4		82.1	
		8.5		8.7
4.80	77.9		73.4	
		8.6		8.9
4.90	69.3		64.5	
		6.9		6.2
5.00	62.4		58.3	
		4.7		4.7
5.10	57.7		53.6	
		3.3		3.2
5.20	54.4		50.4	
	Alcoholic Solutions			
4.40	99.5		98.6	
		1.9		2.1
4.50	97.6		96.5	
		5.0		3.7
4.60	92.6		92.8	
		10.1		9.0
4.70	82.5		83.8	
		14.9		14.3
4.80	67.6		69.5	
		13.6		13.4
4.90	54.0		56.1	
		7.7		8.6
5.00	46.3		47.5	
		3.9		3.9
5.10	42.4		43.6	
		2.0		1.9
5.20	40.4		41.7	

optimum analytical conditions were found by Matuszak and Brown (*14*). From all these investigations it can be concluded that, although the ability to judge the end point can be acquired, it requires considerable experience, the use of a color standard, and much time and patience.

For investigation of this titration a solution of pure sodium fluoride containing 0.04505 gram of fluorine per 100 ml., a 0.04845 *N* solution of thorium nitrate, both 0.1 and 1.0% aqueous solutions of sodium alizarin sulfonate, and a monochloroacetic acid buffer solution 0.2 *M* in both acid and salt of a pH of 3.15 were prepared.

The transmittancy curves of both the yellow form of the indicator and the red thorium lake are shown in Figure 3. Accordingly, filters which had transmittance-band maxima at 420 and 515 mμ were selected.

The use of dextrin, agar-agar, gum arabic, gelatin, and starch as a protective colloid was tried. Of these, only starch was satisfactory and it was found that 10 ml. of a 1% starch solution with 70 ml. of the fluoride solution improved the detection of the color change in aqueous solution (*23*).

The end point of the titration was determined by observing the maximum change in the dial readings of one of the slidewires. A suitable quantity of sodium fluoride solution was added from a buret to a titration cell of 32-mm. light path. This was diluted to about 70 ml. with water, or a mixture of 40 ml. of water and 20 ml. of 95% ethyl alcohol was added. Fifteen drops of a 0.1% indicator solution were added and the color was discharged with 1 drop of 0.1 *N* hydrochloric acid. Then 3.5 ml. of buffer solution were added and the titration was made with 0.05 *N* thorium nitrate. A fixed time interval of 1 or 2 minutes was allowed to elapse between the addition of the thorium nitrate and the adjustment of the rheostat.

With the titration in aqueous solution the gradual precipitation of the thorium fluoride in the vicinity of the end point caused a definite drift of the galvanometer. It was found that the use of ethyl alcohol not only caused rapid precipitation but also increased the magnitude of the change in the dial readings at the equivalence point, which can be seen from Table I and the curves of Figure 3.

Under these conditions the optimum concentration of indicator was found to be 15 drops of a 0.1% solution in 70 ml. of fluoride solution. This is about twice that used by Reynolds and Hill (*19*) and Matuszak and Brown (*14*). The use of 10 drops necessitated the diminishing of the light intensity to prevent fluctuations of the galvanometer and 20 drops broadened the maximum in the curve of dial readings versus volume and caused the results to be farther removed from the stoichiometric values, as also found by Reynolds and Hill.

On the assumption that the rheostats are wound accurately to the nearest 0.1 dial division, that the reaction will permit a precision of 0.1 division in adjustment, and that the smallest change in the vicinity of the end point is greater than 0.1 division, the precision of the determination should be better the smaller the increments in the volume of thorium nitrate solution. All these conditions were not satisfied with this apparatus. With aqueous solutions the difference in changes in the dial readings for 0.05-ml. increments near the end point was of the same order of magnitude as the uncertainty in the dial readings. Although this was not true with alcohol present, still in this case the interpolation of the readings with 0.1-ml. increments gave sufficient precision and increased the speed of titration. The titration can be made with 0.02 *N* as well as 0.1 *N* thorium nitrate solution, although the precision decreases with decreasing concentration.

The effect of varying the amount of fluorine was studied to determine if the relationship between the amounts of fluorine and thorium nitrate was linear over a given range and also whether the results were stoichiometrically exact. The titrations were made both by balancing the circuit with the rheostat in the 420 mμ arm, with the rheostat in the 515 mμ arm fixed, and vice versa. In the first case the position of the dial in the 420 mμ arm is directly proportional to the ratio of the transmittancy of the 515 mμ beam to that of the 420 mμ beam. Because the change in this ratio is assumed to pass through a maximum at the end point, the change in dial readings also passes through a maximum. In the second case the dial readings are inversely proportional to this same ratio, so the change in the reciprocals of the dial readings should pass through a maximum.

Table II. Determination of Fluoride

Detn. No.	F^- Added, Mg.	F^- Found, Mg.	Error, Mg.
1	2.26	2.20	−0.06
2	4.51	4.41	−0.10
3	6.78	6.65	−0.13
4	9.01	8.85	−0.16
5	2.26	2.20	−0.06
6	4.51	4.42	−0.09
7	6.77	6.64	−0.13

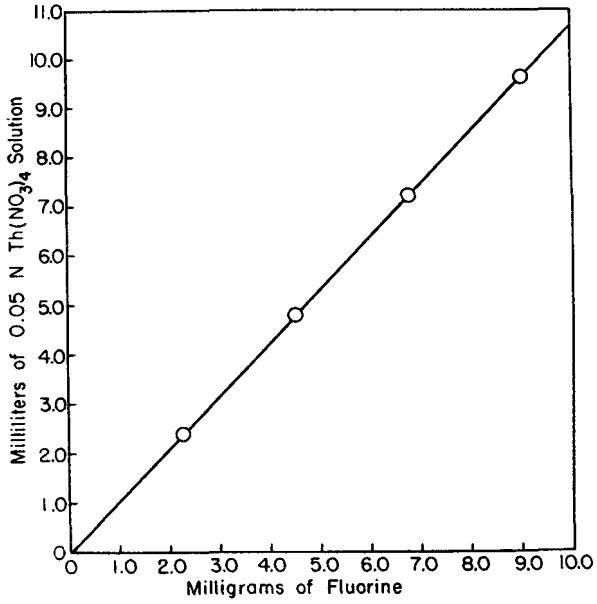

Figure 4. Linearity of Sodium Fluoride–Thorium Nitrate Relationship

The results for varying amounts of fluorine are given in Table II and the linear relationship is shown in Figure 4.

The results are not stoichiometrically exact. However, this is to be expected because of the high indicator concentration used and because it was assumed that the change in transmittancy passes through a maximum at the end point, whereas actually it is the change in absorbency or the logarithms of the transmittancy which does. The second error was calculated and found to be only 0.03 ml., so the main error was due to the indicator. Within the concentration range studied fluorine can be titrated with 0.05 *N* thorium nitrate solution with a precision of ±0.01 ml. and the same accuracy could be attained by applying an indicator correction or by standardizing the thorium nitrate solution by this method.

The method of extrapolating two straight lines to determine the end point was also applied to this titration. Some results are shown in Figure 5.

This method is more time-consuming than the other, the nearly horizontal line is not very clearly defined probably because of incomplete precipitation, coagulation, etc., and the results are not as accurate because of the higher indicator concentration required.

DETERMINATION OF CRITICAL CONCENTRATION OF SOAP SOLUTIONS

Aqueous solutions of most soaps exhibit a more or less abrupt change in physical properties over a relatively short concentration range. This phenomenon has been attributed to the formation of oriented soap aggregates, and the concentration at which it occurs has been termed "the critical concentration for the formation of micelles."

Corrin, Klevens, and Harkins (*8*) were the first to use the alteration of the absorption spectrum of dye solutions to determine the critical concentration. They employed a visual titration based upon the fact that the absorption spectrum of pinacyanol chloride in aqueous solutions of anionic soaps changes sharply to that characteristic of its solutions in organic solvents over a short range of soap concentrations. Corrin and Harkins (*6*) used the same technique for the determination of the critical concentrations of anionic soaps with rhodamine 6G and of cationic detergents with Sky Blue FF, eosin, fluorescein, and acidified 2,6-dichlorophenolindophenol. They (*7*) also measured the depression of the critical concentration by the addition of an electrolyte.

The transmittancy curves for various concentrations of anionic soaps in aqueous pinacyanol chloride solutions (*13*) show that the color change at the critical concentration is the result of a rise in the intensity of the absorption band at 480 mμ and a simultaneous fall in the intensity of two others at about 570 and 615 mμ. Because no absorption spectra of dyes for the titration of cationic detergents were available and because the use of 2,6-dichlorophenolindophenol might introduce an error due to the acid required, Sky Blue FF was chosen.

The sodium dodecyl sulfate was recrystallized twice from ethyl alcohol–ether solutions. The *n*-decyl and *n*-dodecyl trimethyl ammonium bromides were prepared by refluxing the corresponding *n*-alkyl bromides with alcoholic solutions of trimethylamine (*22*). Each soap was recrystallized three times from methanol-ether solutions, and the solvent was removed by vacuum desiccation and stored over phosphorus pentoxide. The potassium bromide was reagent grade. The pinacyanol chloride and Sky Blue FF were used as received from Eastman and National Aniline. A stock solution of sodium dodecyl sulfate was prepared by dissolving 1.900 grams of the soap in 1 liter of 10^{-5} *M* pinacyanol chloride solution. The cationic soap solutions were prepared individually for each determination and a 10^{-5} *M* solution of Sky Blue FF was used.

The spectral behavior of pinacyanol chloride in various concentrations of sodium dodecyl sulfate, shown in Figure 6, agrees with that for other anionic soaps (*8*). Filters with transmittance-band maxima at 490 and 610 mμ were used.

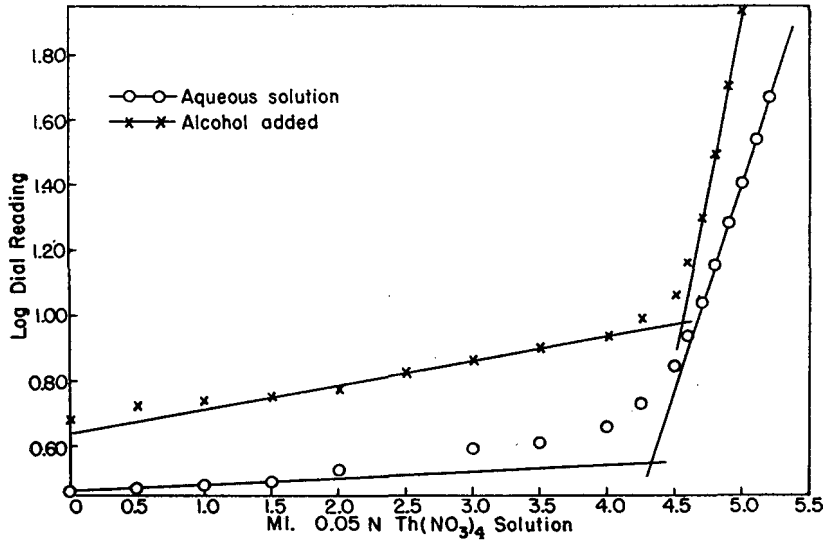

Figure 5. Titration Curves for Intersecting-Line Method

The titration was made by first placing 65 ml. of the stock solution of sodium dodecyl sulfate containing the proper amount of dye in a 32-mm. titration cell. Then the dye solution (same concentration as in sample) was added in 0.50-ml. increments and the rheostat in the 610 mμ arm was adjusted to balance after a fixed time interval of 1 or 2 minutes. The maximum change in the position of the rheostat dial was taken as the end point.

The results in Table III show that equilibrium is more nearly approached for 2-minute than for 1-minute intervals, but the titration is empirical and should always be made under the same conditions. These results agree well with the value of $6.02 \times 10^{-3} M$ obtained by Corrin and Harkins (*6*) by visual titration.

In addition to the fact that the dye solution itself faded very slowly, the spectrum of the dye in the soap solution (Figure 6) apparently was altered on standing, probably owing to the disappearance of the band at 480 mμ.

Spectra for Sky Blue FF in solutions of cationic detergents were not available (*10*), so these were determined for $10^{-5} M$ aqueous solutions as shown in Figure 7.

An examination of these spectra shows that they represent simply a cumulative effect of several close component bands. Nevertheless, it is obvious from these spectra and those determined for several other cationic soaps in solutions of this dye that at the critical concentration a sharp decrease occurs in the intensity of an absorption band whose maximum is about 630 mμ. However, the simultaneous increase in the intensity of any other absorption band is considerably masked. Filters with transmittance-band maxima at 515 and 750 mμ were selected as the best available.

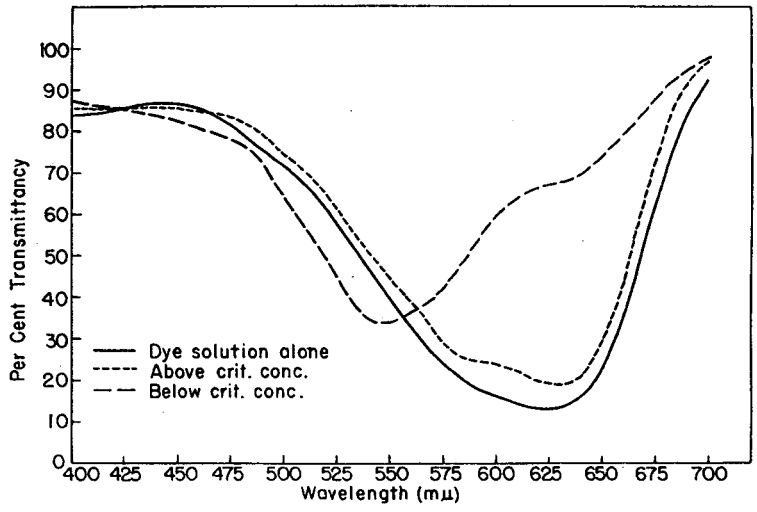

Figure 7. Transmittancy Curves of Sky Blue FF in *n*-Dodecyl Trimethyl Ammonium Bromide

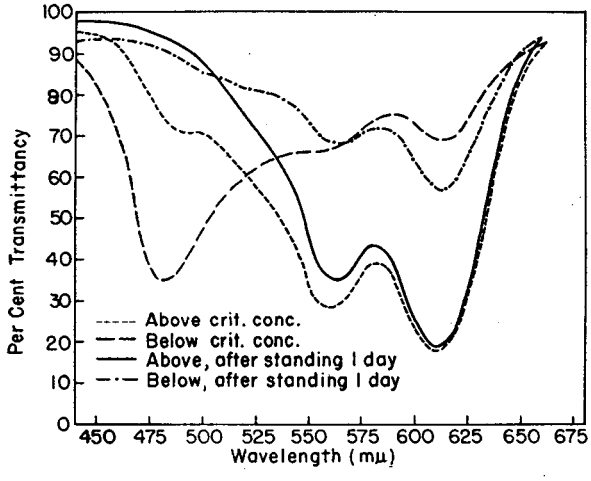

Figure 6. Transmittancy Curves of Pinacyanol Chloride in Sodium Dodecyl Sulfate

Table III. Critical Concentration of Sodium Dodecyl Sulfate

Detn. No.	1-Min. Interval	2-Min. Interval
1	$6.09 \times 10^{-3} M$	$6.18 \times 10^{-3} M$
2	$6.09 \times 10^{-3} M$	$6.18 \times 10^{-3} M$
3	$6.12 \times 10^{-3} M$	...

Table IV. Critical Concentration of Cationic Detergents

Detergent	Wt. of Detergent, Grams	Dye Soln., Ml.	Critical Concn., *M*
n-Decyl	1.3502	12.7	$6.1_9 \times 10^{-2}$
	1.2500	7.7	$6.1_9 \times 10^{-2}$
	1.2200	7.2	$6.0_3 \times 10^{-2}$
n-Dodecyl	0.3100	3.1	1.48×10^{-2}
	0.3010	1.3	1.47×10^{-2}
	0.3000	1.8	1.46×10^{-2}

Titrations were made as with sodium dodecyl sulfate, except that the increments of dye solution were 1.0 ml. and all time intervals were 2 minutes. The results are given in Table IV.

The slightly lower value for the *n*-decyl salt, compared to the value of $6.36 \times 10^{-2} M$ of Corrin and Harkins, may be attributed to the lower dye concentration or a slight impurity in the soap. Inasmuch as no determinations of the value of the *n*-dodecyl salt with Sky Blue FF have been reported, the value obtained must be compared with the value of $1.45 \times 10^{-2} M$ from the conductometric measurements of Scott and Tartar (*22*). Just as in conductometric measurements, the transition occurred over a wider concentration range for the *n*-decyl than for the *n*-dodecyl soap.

SUMMARY

As Mika (*15*) and others have pointed out, although photometric measurements can be used to good advantage for the rapid, routine detection of color changes difficult to find with the naked eye, they increase only the precision, not the accuracy of the result. Thus, in acid-base titrations the accuracy of the result is still susceptible to the errors associated with indicators (salt error, protein error, etc.) as well as factors affecting the pH of the solution directly—e.g., temperature.

The simplicity of the method of titrating to a previously balanced position of the galvanometer and the ease and rapidity with which it may be executed make it especially suitable for routine analyses. It has at least another distinct advantage in that the end point need not be at or near the mid-point of the transition range of the indicator.

This method has been applied to various sorts of acid-base titrations with a precision of about 0.01 ml. in 25 ml.

The speed and accuracy of a phototurbidimetric titration have been shown with the titration of sulfate. The average deviation of three titrations from the correct value of about 25 mg. of sodium sulfate was 0.07 mg. and the maximum deviation was 0.13 mg.

Although it is somewhat empirical, the method of titrating to the maximum change in the dial readings has been suggested for those titrations to which the other methods definitely cannot be applied or as a substitute for the longer intersecting-line methods.

A study has been made of a number of optimum analytical con-

ditions for the photoelectric titration of fluoride with thorium nitrate using sodium alizarin sulfonate. It was found, in spite of previous reports to the contrary, that the end point of this titration is apparently improved by the presence of alcohol. In the range of 2 to 10 mg. of fluoride the average deviation in the volume of thorium nitrate was ±0.01 ml.

Finally, the apparatus has been applied to the visual titration technique, suggested by Harkins, for the determination of the critical concentration for micelle formation in soap solutions by the spectral change of a dye.

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Amperometric Titrations with Ferrocyanide

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Conditions for the amperometric titration of zinc and indium with ferrocyanide are described. The method for zinc depends upon titration in a supporting electrolyte of ammonium acetate that is 1.7 molar at an applied potential of −1.4 volts *vs.* the saturated calomel electrode. The precipitate formed approaches $Zn_2Fe(CN)_6$ in composition. The method for indium depends upon titration in a supporting medium of potassium chloride that is 0.1 molar at an applied potential of −0.75 volt *vs.* the saturated calomel electrode. The precipitate formed has a composition of approximately $In_4[Fe(CN)_6]_3$. Complex formation is suggested as the reason for precipitation of normal ferrocyanides rather than double salts.

THE amperometric titration of ferrocyanide with zinc ion in 0.2 *N* hydrochloric acid solutions at a potential of −1.2 volts *vs.* the saturated calomel electrode was reported by Spalenka (*6*). Spalenka also reported that the reverse titration could be performed in an ammoniacal solution containing ammonium chloride, but that the results were somewhat erratic. The well-known method of titrating zinc with ferrocyanide, as adapted for internal indicators and for potentiometric titration, gives a precipitate with a composition of $K_2Zn_3[Fe(CN)_6]_2$. The method, reported in this paper, of amperometrically titrating zinc in ammonium acetate supporting electrolyte gives a precipitate approaching $Zn_2Fe(CN)_6$ in composition.

The titration of indium with ferrocyanide by a potentiometric method was reported by Bray and Kirschman (*1*). A similar method using diphenylamine as an internal indicator in acetate-buffered solution was reported by Hope, Ross, and Skelly (*2*). The composition of the precipitate given by these investigators was $KIn_5[Fe(CN)_6]_4$. The composition of the precipitate which was found in this investigation was $In_4[Fe(CN)_6]_3$ when the precipitation was performed in 0.1 molar potassium chloride solution.

APPARATUS

The titration vessel was a 150-ml. beaker which was closed with a rubber stopper through which were four holes for entrance of the dropping mercury electrode, the buret tip, the saturated potassium chloride–agar bridge to the saturated calomel electrode (S.C.E.), and the tube to furnish a stream of hydrogen or of nitrogen for removing dissolved oxygen.

The circuit used for making voltage and current measurements was similar to the manual polarograph of Kolthoff and Laitinen (*3*), although some of the titrations were checked, reading the scale manually, on a Sargent Model XII polarograph. Polarograms were run using the Sargent Model XII polarograph.

The buret used for titration was of 10-ml. capacity.

REAGENTS AND METHODS

Stock Solutions. Pure zinc metal was weighed out, dissolved in sulfuric acid, and diluted to volume in a volumetric flask. Pure indium metal was weighed out, dissolved in hydrochloric acid, and diluted to volume in a volumetric flask. Recrystallized potassium ferrocyanide was dried at 105° C. for 24 hours, pulverized, again dried for 24 hours, weighed, dissolved, and diluted in a volumetric flask to prepare a 0.1 molar solution. A small amount of sodium carbonate was added as a preservative. Further dilutions of these stock solutions were prepared by standard volumetric techniques.

Supporting Electrolytes. The ammonium acetate and the potassium chloride used as supporting electrolytes were reagent grade chemicals, found by experiment to give no reduction currents in the range of potential in which they were to be used.

Analytical Methods. In addition to being prepared as accurately as possible, the solutions also were carefully analyzed by volumetric and gravimetric methods.

A standard ceric solution was prepared from primary standard grade ammonium hexanitratocerate and used to check the concentration of the ferrocyanide solution. A thiosulfate solution was standardized by using the ceric solution and was then used to check the stock zinc sulfate solution by the method of Lang (*5*). In this way the ratio of the concentration of the zinc to

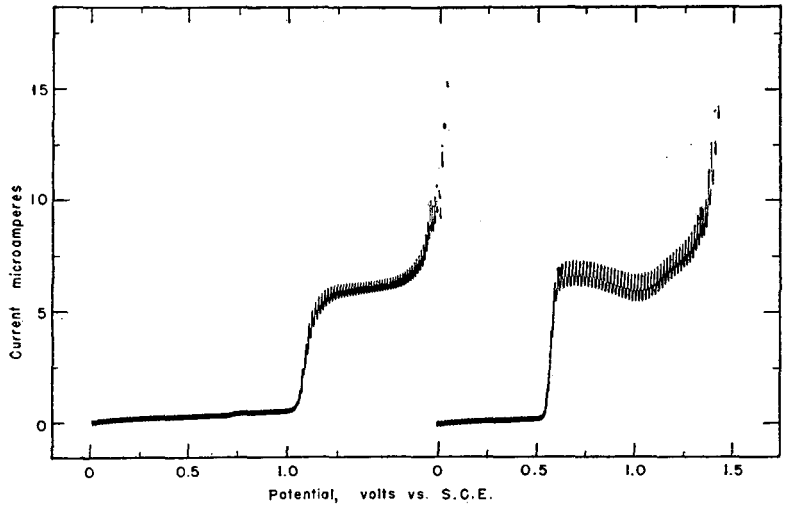

Figure 1. Polarographic Waves for 0.00110 Molar Zinc Sulfate in 1.7 Molar Ammonium Acetate, and 0.000712 Molar Indium Chloride in 0.1 Molar Potassium Chloride

that of the ferrocyanide was determined, using the same primary standard. The results on the stock zinc sulfate solution were as follows: Zinc sulfate solution made up to be 0.3293 molar was found to be 0.3295 molar by gravimetric analysis (precipitating zinc ammonium phosphate), and was found to be 0.3299 molar by using the volumetric method of Lang. The concentration of the indium chloride solution was checked by careful gravimetric analysis, weighing the ignited precipitate as In_2O_3. The indium solution made up to be 0.7125 molar was found to be 0.7120 molar.

Amperometric Titrations. An appropriate quantity of the standard zinc or indium solution to be used was measured into the titration cell. Sufficient supporting electrolyte, gelatin, and water were added to bring the volume to 100 ml. Hydrogen or nitrogen was bubbled through to remove dissolved oxygen, and the titration was begun. Hydrogen or nitrogen was bubbled through the titration cell after each increment of titrant and before making the current readings, to mix thoroughly and to remove the dissolved oxygen added with the titrant. Corrections were applied to the current readings to compensate for dilution and the values were plotted; the end point was obtained by extrapolation.

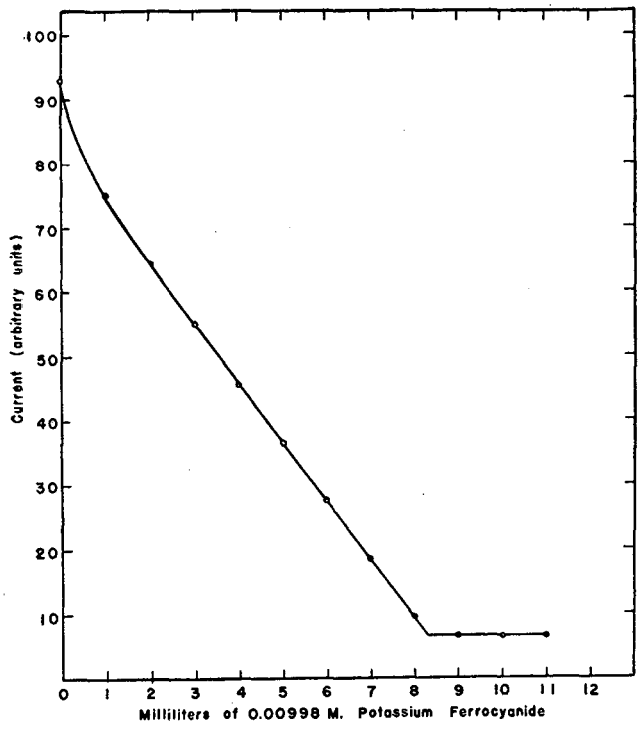

Figure 2. Amperometric Titration of Zinc with Ferrocyanide

50.00 ml. of 0.003299 *M* $ZnSO_4$. End point 8.30 ml.

TITRATION OF ZINC SOLUTIONS

It was found that a well-formed polarographic wave, with half-wave potential −1.12 volts *vs.* the saturated calomel electrode, could be obtained for the reduction of zinc if ammonium acetate solution was used as the supporting electrolyte. Figure 1 shows such a polarographic wave for zinc. By investigation it was found that if the supporting electrolyte was at least 1.7 molar ammonium acetate, reproducible results could be obtained when the titration was performed at a potential of −1.4 volts *vs.* the saturated calomel electrode. A plot of a titration curve for zinc is shown in Figure 2. In some of the titrations, such as one shown in Figure 2, the first two or three points were on a curve, but the points were such that they rapidly approached a straight line, and the plot was easily extrapolated.

Determination of Precipitate Composition. Table I shows the results of the determination of the composition of the precipitate. These data give an average of 1.995 with a mean deviation of 0.003. Thus, one may assume a precipitate composition of $Zn_2Fe(CN)_6$ and make an average error of less than 2.5 parts per thousand. The results reported in Table II are for titrations of various quantities of zinc with solutions of ferrocyanide that are approximately either 0.1 *M* or 0.01 *M*. Inasmuch as only two concentrations of titrant were used, the relative error varies considerably because of the nonoptimum size of some of the titrations. These results are satisfactory for the titration of these amounts of zinc, using a 10-ml. buret for titrations.

Table I. Determination of Composition of Precipitate

(Zinc titrated with ferrocyanide in 1.7 molar solution of ammonium acetate at a potential of −1.4 volts *vs.* S.C.E.)

Zinc, 0.03299 Molar	Ferrocyanide, 0.0998 Molar	Zinc	Ferrocyanide	Ratio
Ml.	*Ml.*	*Millimole*	*Millimole*	
25.00	4.14	0.825	0.413	1.997
25.00	4.15	0.825	0.414	1.994
25.00	4.14	0.825	0.413	1.997
0.003299 Molar	0.00998 Molar			
50.00	8.29	0.1650	0.0827	1.996
50.00	8.25	0.1650	0.0823	2.005
50.00	8.30	0.1650	0.0828	1.993
50.00	8.30	0.1650	0.0828	1.993
50.00	8.32	0.1650	0.0830	1.988
50.00	8.30	0.1650	0.0828	1.993

Table II. Amperometric Titrations of Various Quantities of Zinc with Ferrocyanide

(In 1.7 molar solution of ammonium acetate at a potential of −1.4 volts *vs.* S.C.E.)

Zinc Taken	Zinc Found	Difference
Mg.	*Mg.*	*Mg.*
2.16	2.12	−0.04
4.31	4.28	−0.03
4.31	4.30	−0.01
6.46	6.50	+0.04
6.46	6.57	+0.11
8.17	8.12	−0.05
8.17	8.21	+0.04
8.62	8.58	−0.04
10.79	10.81	+0.02
21.6	21.3	−0.3
43.1	42.9	−0.2
81.7	82.0	+0.3

Attempts to titrate smaller quantities of zinc with an approximately 0.001 molar ferrocyanide solution led to the conclusion that the solubility of the precipitate was so great that it was difficult to determine the end point with precision, although an approximate analysis was possible if the quantity of zinc was above 0.2 mg. In this case the titrations were reproducible within about ±3%.

Twenty-one titrations of 81.7 mg. of zinc were run with 0.0998 *M* ferrocyanide, giving an average of 81.6 mg. found with a mean deviation of 0.3 mg.

TITRATION OF INDIUM SOLUTIONS

In agreement with Kolthoff and Lingane (*4*), the authors found that a good polarographic wave could be obtained for indium when the supporting electrolyte was 0.1 *M* potassium chloride. The half-wave potential was found to be −0.56 volt *vs.* the saturated calomel electrode. The second polarographic wave shown in Figure 1 was taken under these conditions. A plot of a typical titration curve for indium at −0.75 volt *vs.* the saturated calomel electrode is shown in Figure 3. The first point is seen to be high, similar to the plot for zinc. The curvature extended further for the more concentrated solutions, but points could be taken closer to the end point in these cases without interference from the solubility of the precipitate. Good end points were consequently obtained.

Determination of Precipitate Composition. Table III shows the results of the determination of the composition of the precipitate. The average of these nine determinations gives a ratio of 1.332 with a mean deviation of 0.003. For subsequent determinations a precipitate composition of $In_4[Fe(CN)_6]_3$ is assumed at the equivalence point.

Table IV shows the results of the titrations of various quantities of indium with ferrocyanide solutions of concentration approximately 0.1 *M* and 0.01 *M*. More dilute indium solutions were titrated with 0.001 *M* ferrocyanide, with essentially the same results that were obtained on zinc. Some of the results in Table IV show moderately large relative errors, due to the fact that only two concentrations of titrant solution were used.

Table III. Determination of Composition of Precipitate

(Indium titrated with ferrocyanide in 0.1 molar solution of potassium chloride at a potential of −0.75 volt *vs.* S.C.E.)

Indium, 0.07120 Molar	Ferrocyanide, 0.0998 Molar	Indium	Ferrocyanide	
Ml.	*Ml.*	*Millimole*	*Millimole*	Ratio
10.00	5.34	0.712	0.533	1.336
10.00	5.35	0.712	0.534	1.334
10.00	5.37	0.712	0.536	1.329
0.00356 Molar	0.00998 Molar			
25.00	6.71	0.0890	0.0670	1.328
25.00	6.71	0.0890	0.0670	1.328
25.00	6.68	0.0890	0.0667	1.335
25.00	6.68	0.0890	0.0667	1.335
25.00	6.70	0.0890	0.0669	1.330
25.00	6.68	0.0890	0.0667	1.335

Table IV. Amperometric Titrations of Various Quantities of Indium with Ferrocyanide

(In 0.1 molar solution of potassium chloride at a potential of −0.75 volt *vs.* S.C.E.)

Indium Taken	Indium Found	Difference
Mg.	*Mg.*	*Mg.*
2.04	2.02	−0.02
4.09	4.09	0.00
6.13	6.09	−0.04
8.17	8.24	+0.07
10.21	10.26	+0.05
16.3	16.2	−0.1
24.6	24.9	+0.3
24.6	24.3	−0.3
44.0	44.3	+0.3
77.1	76.4	−0.7
81.7	81.6	−0.1

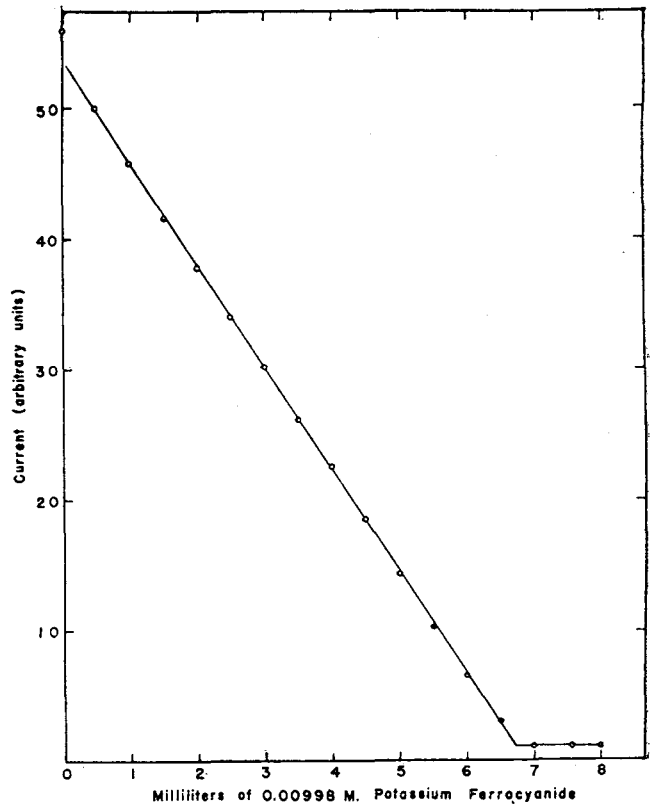

Figure 3. Amperometric Titration of Indium with Ferrocyanide

25.00 ml. of 0.00356 *M* $InCl_3$. End point 6.71 ml.

Thus, some of the titrations involved volumes that were far from the optimum. These results are satisfactory under the conditions of the titration.

RESULTS

It was found that quantities of either zinc or indium from 2 to 80 mg. could be determined by an amperometric titration method, using potassium ferrocyanide as the titrant. If the titrant solution had a concentration which would give an optimum size titration, an accuracy of better than ±0.5% error could be obtained. The precipitate composition for zinc was shown to approximate $Zn_2Fe(CN)_6$ at the end point, while for indium the composition of the precipitate approached $In_4[Fe(CN)_6]_3$, both within 0.25% (relative).

The application of one of these titrations to the determination of zinc or indium in an alloy would involve separations to remove from the zinc or indium other metals that would precipitate with ferrocyanide. For determination of zinc in brass this would involve removal of tin, lead, copper, nickel, and iron in the usual manner and titration of the zinc remaining. For determination of indium in a dental alloy the necessary separations are outlined by Hope, Ross, and Skelly (*2*). The precipitated indium hydroxide could be dissolved in hydrochloric acid and titrated with ferrocyanide, by following the method given in this paper.

The fact that a different precipitate composition was obtained in the precipitation of zinc than in the potentiometric method of titration is not surprising, because this investigation used a moderately concentrated ammonium acetate solution which is an entirely different electrolyte situation than that used in the other case. The zinc ions are known to form ammonia complexes, and in 1.7 molar ammonium acetate some complexing would be expected. The fact that a half-wave potential of

−1.12 volts *vs.* the saturated calomel electrode was observed, instead of −1.01 volts, is evidence of some kind of complexing, not all of which can be explained by ammonia complexing. This may indicate that the acetate ion also has a tendency to form complexes with the zinc.

The previous investigators (*1, 2*) state that their methods for indium can tolerate no chloride ion, whereas in the procedure used in this investigation the chloride ion is present to the extent of 0.1 molar. This fact may explain the different precipitate composition obtained, for it is well known that indium chloride is highly complexed and under these conditions the authors have found the normal indium ferrocyanide to precipitate.

The conclusion that might be drawn from this situation is that the presence of complex ions, in both of these cases, must account in some way for the precipitation of normal ferrocyanides rather than the potassium double salts, although potassium ions are added during the titrations.

ACKNOWLEDGMENT

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Polarographic Study of Lower Nitroparaffins in Nonalkaline Media

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Half-wave potentials for nitromethane, nitroethane, 1-nitropropane, and 2-nitropropane in well-buffered aqueous solutions have been determined as a function of pH at one concentration. For each compound $E^1/_2$ was found to be a linear function of pH at a molar concentration of about 2×10^{-4}. Equations for each of these linear relationships have been formulated. Diffusion currents have been determined for nitromethane at four different concentrations and two pH values, and for 1-nitropropane at six different concentrations and three pH values. For each compound the linear relationship expected from the Ilkovič equation holds between concentrations of about 2×10^{-3} and 6×10^{-5} molar at each pH. The irreversibility and the nature of the end products of the reduction have been confirmed. The type and strength of the supporting electrolyte used are indicated as being significant.

TO DATE, aliphatic nitro compounds have not been extensively investigated polarographically. DeVries and Ivett (*5*) have studied the polarographic reduction of six nitroparaffins dissolved in 0.05 *M* sulfuric acid and 0.05 *M* sodium sulfate. A polarographic method for estimating the amount of nitromethane in air has recently been developed (*11*), but here again unbuffered solutions were used. The pseudo acid properties of some nitroparaffins, including the determination of rates of nitro-aci tautomerism in buffered alkaline solutions, have been polarographically investigated (*9*). The only other study of the polarographic reduction of nitroparaffins in buffered solutions has been that of Petru (*10*), using 30% methanol solutions in Britton-Robinson buffers (*2*). In the present work aqueous solutions of nitroparaffins buffered with Clark and Lubs mixtures (*8*) were used. Presumably because of a different solvent and/or buffer system, data reported herein differ from those recorded elsewhere.

EXPERIMENTAL

Apparatus. The instrument used was a Leeds & Northrup Electrochemograph, Model 42200-A1. Errors resulting from the inherent lag in the recorder unit of this instrument were eliminated, when necessary, by manual operation.

Solutions studied were contained in a 2-ounce, screw-top bottle, thermostated at 25° ± 0.1° C.

Six-inch (15-cm.) capillaries of "marine barometer tubing" were connected to the mercury reservoir with Tygon tubing. The capillary characteristics are reported with each analysis.

Connection from the polarographic cell to the reference electrode was made through a saturated potassium chloride salt bridge. Resistance from the cell through the reference electrode was kept below 500 ohms at all times. Throughout the work a saturated calomel electrode was used as reference; thus, all voltages given in the results are *vs.* the saturated calomel electrode.

Materials Used. The nitroparaffin samples employed were kindly furnished by the Commercial Solvents Corporation. Water-white and declared as better than 99% pure, they were used without further purification. Stock solutions of these compounds were prepared, from which samples could be pipetted for analyses.

The concentrations of these stock solutions were such that 1-ml. aliquots could be added to 40 ml. of deoxygenated buffer solutions to give the desired nitroparaffin concentrations. It was determined beforehand that the addition of a slight amount of dissolved oxygen in the 1-ml. aliquots does not appreciably affect the results. Deoxygenation was accomplished by the use of purified tank nitrogen.

The pH of all buffers were checked just before and frequently during use.

Determination of Polarograms. In all this work only one reduction wave was found, and no maxima were encountered. A modification of one of the four methods suggested by Borcherdt *et al.* (*1*) to measure polarograms was used. Resistances through the cell circuit for finding iR values, and drop times for finding $m^{2/3}t^{1/6}$ values were measured at applied potentials as near the half-wave potentials as possible (current flowing). The iR drops were subtracted from the measured half-wave potentials for corrected values. Two runs on each of two solutions were

Table I. Half-Wave Potentials as Function of pH

pH	Nitromethane[a]	Nitroethane[b]	1-Nitropropane[c]	2-Nitropropane[c]
2.0	−0.714	−0.680	−0.649	−0.721
3.0	−0.750	−0.742	−0.705	−0.774
4.0	−0.780	−0.783	−0.754	−0.816
5.0	−0.798	−0.822	−0.792	−0.837
6.0	−0.846	−0.878	−0.843	−0.883
7.0	−0.881	−0.910	−0.887	−0.909

$m^{2/3}t^{1/6}$ is: [a] 1.74 mg.$^{2/3}$sec.$^{-1/2}$; [b] 1.79 mg.$^{2/3}$sec.$^{-1/2}$; [c] 2.70 mg.$^{2/3}$ sec.$^{-1/2}$.

Table II. Equations of $E^1/_2$ − pH Curves

	Slope, Mv./pH	$-E^0$, Volt (Apparent)	Equation
Nitromethane	32	0.650	$-E^1/_2 = 0.650 + 0.032$ pH
Nitroethane	45	0.600	$-E^1/_2 = 0.600 + 0.045$ pH
1-Nitropropane	45	0.565	$-E^1/_2 = 0.565 + 0.045$ pH
2-Nitropropane	37	0.658	$-E^1/_2 = 0.658 + 0.037$ pH

usually made for each determination. Deviation from a mean value was usually well under 1%.

RESULTS AND DISCUSSION

Dependence of $E^1/_2$ on pH. The half-wave potentials reported in Table I were obtained as the result of electrolyses of solutions about 2×10^{-4} M in the nitroparaffin. The increase in negative half-wave potentials with increasing pH is in agreement with the fact that in the reduction of most organic compounds hydrogen ions are involved in the electrode reactions, and so as the acidity increases the reductions become easier and the half-wave potentials are more positive.

It has been shown (*7*) that to a close approximation the half-wave potential of the general electrode reaction for organic substances at a given concentration is given by

$$E^1/_2 = E^0 - 0.0591 \text{ pH} \qquad (1)$$

if the electrode reaction is reversible. Table I shows that, in accord with Equation 1, the half-wave potentials for each of the four nitroparaffins are approximately linear functions of pH, even though in these cases the reactions are irreversible.

Quantitatively, Equation 1 also indicates that for each unit increase in pH the half-wave potential should become 59.1 mv. more negative, and that the intercept of the $E^1/_2$ − pH curve would give a value for E^0. Equations corresponding to Equation 1 for each compound as determined from such $E^1/_2$ − pH curves are given in Table II. If a reaction at the dropping mercury electrode depends directly upon the square root of the hydrogen ion concentration, the slope of the $E^1/_2$ − pH curve would be 29.55 mv. per pH unit. The reduction of nitromethane approaches this condition. The slopes of the curves for nitroethane and 1-nitropropane indicate that the reductions of these compounds depend upon the hydrogen ion concentration raised to the three-fourths power, while that of 2-nitropropane is intermediate. It is interesting to note that Kolthoff and Liberti (*6*) found the reduction of nitrosophenylhydroxylamine depended upon the square of the hydrogen ion concentration.

Dependence of I_d on Concentration. Diffusion currents have been determined for nitromethane buffered at pH 3 and pH 6 at four different concentrations; and for 1-nitropropane buffered at pH 3, pH 5, and pH 7 at six concentrations. The linear relationship expected on the basis of the Ilkovič equation was found to hold between molar concentrations of approximately 2×10^{-3} and 6×10^{-5} for each compound. At lower concentrations of 1-nitropropane the reduction waves are not at all well defined and the limiting current plateaus were difficult to evaluate.

The slight differences which were found in diffusion currents at the same concentrations and pH's for nitromethane and 1-nitropropane were undoubtedly due to differences in diffusion coefficients. Inasmuch as 1-nitropropane is composed of larger molecules and thus would diffuse more slowly through a given solution than would nitromethane, reductions of the latter compound would be expected to result in higher currents. Such was seen to be, in general, the case. Additionally, because the diffusion currents for each compound were of the same order of magnitude, the reduction of each compound involves the same number of electrons.

Reduction Process. The irreversibility of the reduction of the nitro group at the dropping mercury cathode has been demonstrated by many investigators, and is confirmed in the authors' work. For example (*7*), in every instance they have found that ΔE applied/$\Delta \log \frac{i}{I_d - i}$, determined graphically, corresponded to a value between 0.54 and 0.66 for n, the number of electrons involved in the reduction. Furthermore, for reversible reactions $E^1/_2$ is independent of changes in concentration (*7*). Table III shows that for nitromethane, at least, this is certainly not the case.

That the reduction of nitroparaffins at the dropping mercury cathode forms the corresponding hydroxylamines (*10*) has also been confirmed here by means of the Ilkovič equation. By using the Stokes-Einstein diffusion equation and the Nernst expression (*7*), and assuming that molecules of nitromethane are of about the same size as those of acetic acid, the diffusion coefficient of nitromethane at infinite dilution is estimated to be 1×10^{-5} sq. cm. per second. When this value along with experimentally determined values for the other variables was used in the Ilkovič equation, values for n of from 3.7 to 5.0 resulted, and in most instances a reduction involving 4 electrons was indicated. (This variation is due, in part at least, to the fact that the diffusion coefficient for nitromethane, as estimated above, undoubtedly does not remain constant with changing pH.)

Table III. Nitromethane Half-Wave Potentials as a Function of Concentration

Molar Concn.	pH 3.0	pH 6.0
2.439×10^{-3}	−0.733	−0.834
6.0975×10^{-4}	−0.761	−0.842
2.439×10^{-4}	−0.764	−0.849
6.0975×10^{-5}	−0.791	−0.849

Table IV. Half-Wave Potentials and Diffusion Currents as a Function of Indifferent Electrolyte at pH 4.0

Buffer	Nitromethane $-E^1/_2$, volt	Nitromethane I_d, μa.[a]	Nitroethane $-E^1/_2$, volt	Nitroethane I_d, μa.[a]
Clark and Lubs	0.781	3.3	0.775	2.9
MacIlvaine[b]	0.742	3.3	0.726	3.0
Acetate[c]	0.734	3.3	0.726	2.9
Benzoate[d]	0.796	3.2	0.754	2.9

[a] All diffusion currents are referred to same $t^{1/6}$ value of 1.33 sec.$^{1/6}$ at applied mercury pressure of 60 cm. Value for m is 1.56 mg./sec.
[b] (*3*).
[c] Solution ca. 0.5 molar with respect to acetic acid.
[d] Solution ca. 0.0125 molar with respect to benzoic acid.

Effect of Indifferent Electrolyte. That the type and strength of supporting electrolyte used in polarographic work involving the nitroparaffins have a decided effect on their half-wave potentials can readily be seen by comparing the results presented in this paper with those of Petru (*10*) and Miller *et al.* (*9*). Solutions used by the former were mentioned at the beginning of this paper. The latter authors employed double strength MacIlvaine disodium phosphate–citric acid buffers in the pH range 2 to 7. Although half-wave potentials do not remain constant with changing concentration (Table III), accounting in part for the discrepancies, inasmuch as somewhat different nitroparaffin concentrations were used by the present authors than by others, the effect of the indifferent electrolyte is important, as can be seen below.

Table IV shows the half-wave potentials resulting from the electrolyses of 2.439 × 10^{-4} molar nitroparaffin solutions, all buffered at pH 4.0. The diffusion currents for each remained constant, within the limits of experimental error, regardless of the buffer solution employed. However, each compound is more easily reduced in the citric acid–disodium phosphate buffer or in the acetate buffer than in either the benzoate or phthalate buffers. Although no explanation of this phenomenon can be given here, it seems logical that these differences may be due to adsorption phenomena at the dropping mercury cathode, or to possible differences in the tautomeric equilibria of the nitroparaffins in different media. In any event, it is clear that the type and concentration of buffer used must be given in order for $E^1/_2$ to have significance. The dependence of the polarographic reduction of aromatic nitro groups on the buffer system has already been suggested (*4*).

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Spectrophotometric Determination of Ruthenium

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A method is described for the determination of ruthenium by measuring the absorbancy of a potassium ruthenate solution at 465 mμ. The range of the method is from 1 × 10^{-4} to 4 × 10^{-4} molar ruthenate in 2 *M* potassium hydroxide. The 95% confidence interval is ±3% of the ruthenium present, with no interference from the common anions. The other platinum metals do not interfere in amounts equal to one fifth of the ruthenium concentration.

IN CONNECTION with the preparation of ruthenium compounds (*2*), an analysis of a large number of samples was required. The determination of ruthenium in these samples by the gravimetric method of Gilchrist and Wichers (*6*) proved to be entirely satisfactory from the standpoint of accuracy. However, the time required and the tedium involved in the procedure prompted an investigation of spectrophotometric methods. The methods of DeFord (*5*) and Breckenridge and Singer (*3*) were considered too sensitive for this application. Other spectrophotometric methods have been proposed (*12*), but they have not been developed sufficiently for general application.

In preliminary investigations several different ruthenium compounds were fused with a mixture of potassium hydroxide and potassium nitrate (*7*, *14*). In every case a clear orange-red solution was obtained when the melt was dissolved in water. The intensity of the color and the fact that it absorbed strongly at 465 mμ wave length suggested its use for the estimation of ruthenium. Silverman and Swartout (*13*) also found the maximum absorbancy of ruthenate at 465 mμ.

This paper is a report of a study made on the use of potassium ruthenate solution as a basis for the spectrophotometric determination of ruthenium. The terminology and symbols used in the discussion of spectrophotometric properties of solutions are those suggested by the National Bureau of Standards (*10*). The statistical terminology used is that of Brownlee (*4*). All precision figures attached to experimental results are given as the 95% confidence interval.

EXPERIMENTAL

Reagents. Reagent grade potassium hydroxide pellets and crystalline potassium nitrate were used.

Apparatus. All absorbancy measurements were made with a Beckman Model DU spectrophotometer, using matched Corex cells of 1-cm. solution thickness. The accuracy of the wave-length drum was verified by means of the sodium *D* line. Fusions were carried out in a gold dish, 30 mm. in diameter, 8 mm. deep, and of 0.50-mm. stock.

Standard Compounds. A solid (ammonium chlororuthenate), approximating the formula $(NH_4)_2RuCl_6$, was prepared according to the procedure of Rogers, Beamish, and Russell (*11*), and analyzed gravimetrically for ruthenium by reduction under hydrogen. This standard contained 29.90 ± 0.04% ruthenium (atomic weight 101.7) after reaching equilibrium in a phosphorus pentoxide desiccator. The composition of this solid varies with the method of preparation.

Ruthenium dioxide ($RuO_2.xH_2O$) was made by distilling ruthenium tetroxide into a 10% solution of hydrogen peroxide and refluxing the distillate on a steam bath for 1 hour. The brownish-black solid was brought to equilibrium in a desiccator after being ground in an agate mortar. By gravimetric analysis the compound contained 67.70 ± 0.04% ruthenium.

Ammonium nitroso pentachlororuthenate, $(NH_4)_2RuCl_5(NO)$, was obtained from Gilchrist of the National Bureau of Standards, and was dried over phosphorus pentoxide before being analyzed in this laboratory by reduction under hydrogen. The results, 29.70 ± 0.03% ruthenium, are in good agreement with the stated value of 29.66%.

Metallic ruthenium, obtained from the reduction of the above compounds under hydrogen, was analyzed spectrographically. Copper, iron, and silicon were present, but in no case did the total of these exceed 0.01% of the sample weight. None of the other platinum metals were detected.

General Procedure. The ruthenium metal, or compound equivalent to approximately 8 mg. of ruthenium, was covered with 1 gram of potassium nitrate and 0.3 gram of potassium hydroxide in a gold dish. The dish was carefully heated until the hydroxide just melted and wet the sample. Any reaction that occurred at this point was allowed to proceed slowly until evolution of gases ceased, and then the nitrate was carefully melted. After the water was eliminated and the melt had become quiescent, the dish was heated to a dull redness for 20 to 30 seconds. The cooled melt was dissolved in water and enough potassium hydroxide was added to make the final volume (usually 250 ml.) 2 *M* in hydroxide. This solution was cooled to 25° ± 1° C. and diluted to exact volume, and the absorbancy was determined immediately at 465 mμ against a water blank.

Experimental Results. Figure 1 is a plot of the logarithm of absorbancy of ruthenate solutions versus wave length (*9*). Measurements were made at intervals of 10 mμ beginning at 320 mμ, and using a nominal spectral band width of 5 mμ.

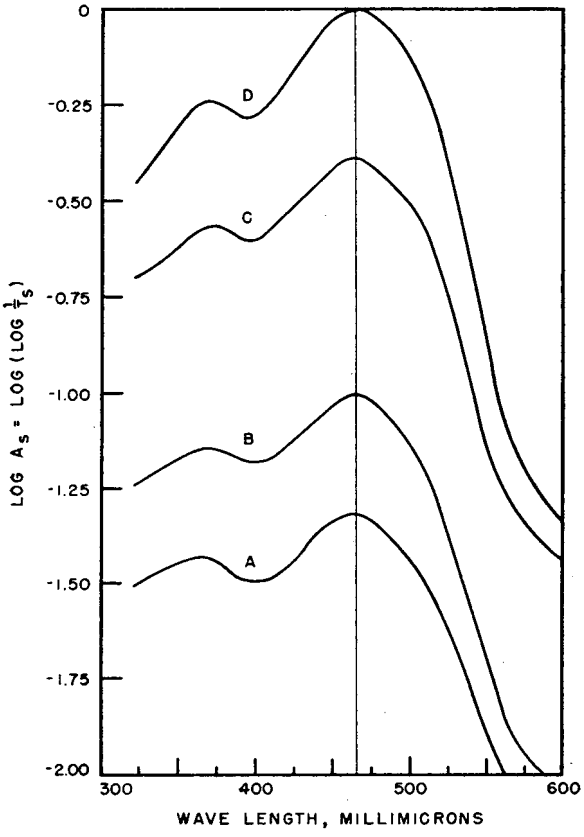

Figure 1. Spectral Absorption Characteristics of Potassium Ruthenate in 2 *M* Potassium Hydroxide Solution

A. 0.265 × 10^{-4} *M* (mole RuO_4^{--}/liter). *B.* 0.56. *C.* 2.25. *D.* 5.60

Table I summarizes the results of the application of the procedure to the standard ruthenium compounds. Values of the molar absorbancy index, a_m, are calculated from measurements of the absorbancy at 465 mμ in the concentration range 1 × 10^{-4} to 4 × 10^{-4} *M* (molar) ruthenate. The molar absorbancy index is defined by the Beer-Bouguer relation:

$$\log_{10} (T_{soln.}/T_{solv.}) = - a_m C b \quad (1)$$

$$\log_{10} 1/T_s = A_s = a_m C b \quad (2)$$

where A_s is the absorbancy, T_s is the transmittancy, C is the (analytical) concentration of the active component in moles per liter, and b is the thickness of the solution measured in centimeters. In Table I, S_m is the standard deviation of a_m, and Y is a parameter defined below.

Figure 2 is a Beer's law plot of A_s measured at 465 and 370 mμ versus concentration. The values of A_s in this plot were obtained from solutions made by fusing ruthenium metal.

DISCUSSION

Fusion of Ruthenium and Its Compounds in Alkali-Nitrate Flux. In the work reported here a mixture of 1 part of potassium hydroxide to 3 parts of potassium nitrate by weight was found to give complete conversion of ruthenium metal and its compounds to the ruthenate. An excess of potassium hydroxide over a 1 to 3 mixture causes spattering of the melt and does not materially aid in the solution of the sample.

The melt becomes greenish black as the sample is attacked and retains this color until brought into contact with moisture. The oxidation of ruthenium probably proceeds in part to the perruthenate state ($KRuO_4$) which yields the ruthenate by reaction with hydroxide in solution (*7*):

$$2KRuO_4 + 2KOH \longrightarrow 2K_2RuO_4 + H_2O + {}^1/_2O_2 \quad (3)$$

Hillebrand and Lundell (*8*) caution that the ruthenate may not dissolve completely from the melt. This difficulty was not encountered in this work, but not more than 12 mg. of ruthenium were present in any one fusion.

Spectral Absorption Characteristics. From Figures 1 and 2 it is evident that a wave length of 465 mμ is best suited for the measurement of the absorbancy of ruthenate solutions. At 370 mμ the absorbancy measurements are scattered and are not represented by a simple curve. At 465 mμ and in the concentration range 1 × 10^{-4} to 4 × 10^{-4} *M* the ruthenate solution follows Beer's law very well. The upper limit was chosen to limit the concentration range to the region in which the most accurate measurements can be made (*1*). Below a concentration of 1 × 10^{-4} *M*, the ruthenate solutions deviate from Beer's law. This range was not investigated thoroughly because ade-

Table I. Molar Absorbancy Index of Potassium Ruthenate in 2 *M* Potassium Hydroxide

Compound	No. of Fusions	a_m	Y	S_m
Ru Metal	17	1728	0.005	9
$(NH_4)_2RuCl_6$	9	1757	−0.003	15
$(NH_4)_2RuCl_5(NO)$	14	1755	0.000	10
RuO_2	5	1752	−0.002	13

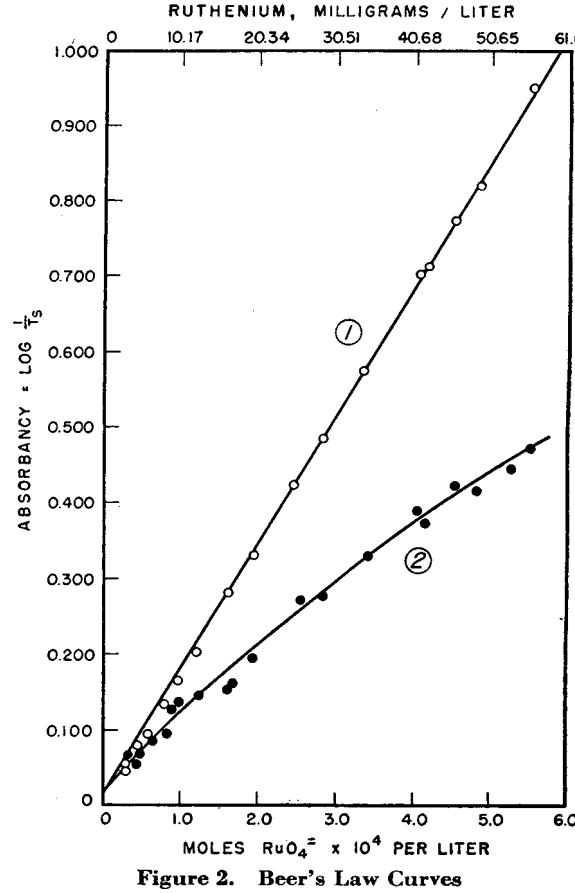

Figure 2. Beer's Law Curves

1. At 465 mμ. 2. At 370 mμ. 1-cm. solution thickness, band width 10 mμ

quate methods are available for the determination of these small amounts of ruthenium (*3*, *5*).

Stability of Potassium Ruthenate Solution. Potassium ruthenate solutions are essentially unstable, but they can be stabilized for at least 0.5 hour by making the solution 2 *M* in potassium hydroxide. Most reducing agents cause immediate precipitation of a black solid from ruthenate solutions regardless of alkalinity. The solution must be protected from dust and grease.

Interferences. The only interferences investigated were those that would arise from the other platinum metals (osmium, iridium, platinum, palladium, and rhodium) or the common anions fluoride, chloride, phosphate, and sulfate.

A sample of each of the platinum metals was carried through the fusion and a spectral absorption curve was made on the solution of the melt. Only osmium metal fused easily, yielding a yellow solution in 2 *M* hydroxide. Twenty milligrams of platinum, iridium, palladium, and rhodium were not completely dissolved by the fusion. None of the resulting solutions absorbed appreciably at wave lengths longer than 400 mμ. At least one compound of each platinum metal was carried through the procedure with the same negative results.

A known amount of ruthenium standard was mixed with a sample of each platinum metal, so that the ratio of ruthenium to the other metal was 5 to 1. This mixture was fused and analyzed for ruthenium. In no case did the analysis differ from the expected value by more than 2%. The procedure has not been applied to alloys of ruthenium with the other platinum metals.

Several analyses were made on ruthenium standards after adding 0.5 gram of the sodium and ammonium salt of one of the common anions to the fusion mixture. In no case did the analysis differ by more than 2% from the known ruthenium content. The absorbancy of these solutions was not affected by adding 0.1 mole per liter of any of these anions to the solution of the melt.

Precision of Method. Although conformity of a system to Beer's law is not an essential characteristic for application to analytical purposes, it is a convenience worth establishing and provides the investigator with a valuable statistic. Continual reference to a calibration curve is eliminated and instead the concentration can be calculated from a simple equation. The statistical treatment given below establishes the validity of the linear relation between absorbancy and concentration and the reliability of the method.

An analysis of variance and covariance of the data summarized in Table I showed no significant differences among the four standards in the linear relation of absorbancy and concentration.

The relation between absorbancy and concentration was assumed to be of the form:

$$A_s = Y + a_m + r \qquad (4)$$

where Y and a_m are parameters to be established and r is a random variate, the distribution of which is assumed to be normal with a mean of zero and unknown variance. Table I shows these regression coefficients for each standard used. The random variate does not differ significantly from zero.

Considering the data collectively, the least square estimate of the straight-line portion of Figure 2, in the concentration range 1×10^{-4} *M* to 4×10^{-4} *M*, is represented by:

$$A_s = 0.0018 + 1742\,C \qquad (5)$$

The standard deviation of Y in this expression is 0.0015. Hence Y does not differ significantly from zero and the straight line can be considered to pass through the origin, indicating negligible absorption by the reagents. The slope of this straight line is the molar absorbancy index. The 95% confidence interval for this slope is 1742 ± 12. The absorbancy increases by 0.174 ± 0.001 unit for every increase of 1×10^{-4} mole per liter in ruthenate concentration.

The molar absorbancy index can be considered a constant with the indicated precision only in the specified concentration range. The numerical value of a_m is useful only for the instrument used and the experimental conditions existing during this investigation.

After the calibration curve has been established as in Figure 2, it is used in either of two ways. The absorbancy of a solution having been determined, the estimated concentration, C_x, may either be read from the plot or calculated from the relation:

$$C_x = 10^{-4}(5.742A_s - 0.0102) \qquad (6)$$

In either case the variance of the slope of the line appears in the estimated concentration. The standard deviations, S_c, and the 95% confidence intervals of the estimated concentrations for various absorbancies are given in Table II. The values of A_s were taken at random and the C_x values calculated from Equation 6. The confidence intervals are calculated from the expression:

$$L = C_x \pm tS_c \qquad (7)$$

$$L_{95} = C_x \pm 2.017\,S_c \qquad (8)$$

where t is Student's t.

From Table II it is evident that in the concentration range 1×10^{-4} to 4×10^{-4} *M* ruthenate, the 95% confidence interval for an estimated concentration can be taken to be not greater than $\pm 3.0 \times 10^{-6}$ *M* ruthenate.

Table II. Standard Deviations and Confidence Intervals for Estimated Concentration

A_s	Estimated Concn. RuO_4^{--}, Moles/Liter $\times 10^{-4}$	Standard Deviation, $S_c \times 10^{-6}$	95% Confidence Interval, $\times 10^{-6}$
0.200	1.138	1.473	±2.9
0.400	2.287	1.414	±2.85
0.600	3.435	1.464	±2.95
0.700	4.009	1.527	±3.08

ACKNOWLEDGMENT

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Total Hardness in Water

Stability of Standard Disodium Dihydrogen Ethylenediamine Tetraacetate Solutions

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Standard solutions of disodium dihydrogen ethylenediamine tetraacetate are being extensively used in determinations of hardness in water. The stability of these solutions is shown to be satisfactory at pH 5 in 1-gallon soft-glass bottles. Calcium chloride solutions are shown to be stable also.

THE determination of total hardness in water using disodium dihydrogen ethylenediamine tetraacetate (disodium dihydrogen Versenate) was devised by Schwarzenbach and reviewed and further developed by Diehl, Goetz, and Hach (*1*). The sodium Versenate forms soluble complex ions with calcium and magnesium. The water sample is brought to a pH of 10 with ammonium hydroxide–ammonium chloride buffer and a few drops of an alcohol solution of eriochromeschwartz T are added as indicator. This dye forms a wine-red, slightly dissociated compound with the magnesium ion. As the titration with the standard sodium Versenate solution proceeds, first the calcium is tied up as a very slightly dissociated compound, the free magnesium is then similarly tied up, and finally, at the end point, the magnesium in the wine-red magnesium–eriochromeschwartz T compound is extracted, causing a sharp change in color from wine red to clear blue.

To ensure an adequate concentration of magnesium in the solution being titrated to form the magnesium–eriochromeschwartz T indicator colored complex ion, magnesium chloride is added to the sodium Versenate solution before it is standardized. Thus, magnesium is automatically added during the titration without the need for a blank correction.

Because the sodium Versenate method of determining total hardness in water is receiving wide commercial acceptance, the present study was made to determine the stability of the standard solutions employed.

PREPARATION OF SOLUTIONS

Standard Sodium Versenate Solutions. A standard sodium Versenate solution was prepared by dissolving about 16 grams of disodium dihydrogen Versenate (obtained from Hach Chemical Company, Ames, Iowa) in 4 liters of distilled water and adding 20 ml. of 0.1 *M* magnesium chloride solution. The pH of this solution was 4.25. Six such solutions were prepared and then the pH of each was adjusted by the addition of the proper amount of sodium hydroxide, so that a series of solutions having a pH of 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 was obtained.

These solutions were placed in sealed soft-glass bottles and their strength was checked against a standardized solution of calcium chloride at the indicated time intervals.

Secondary Standard Calcium Chloride Solution (permanent comparison standard). About 16.6 grams of calcium chloride dihydrate were dissolved in 12 liters of distilled water and stored in a soft-glass carboy fitted with an Ascarite–carbon dioxide trap.

Primary Standard Calcium Chloride Solution. Exactly 1 gram of special primary standard grade calcium carbonate was dissolved in dilute hydrochloric acid, boiled, and, after cooling, diluted to 1 liter.

Buffer Solution. To 67.5 grams of ammonium chloride (A.C.S. specification grade) were added 570 ml. of concentrated ammonium hydroxide. After mixing the solution was diluted to 1 liter.

Indicator Solution. Five grams of ManVer indicator powder (obtained from Hach Chemical Company, Ames, Iowa) consisting of 1 part of analytical grade eriochromeschwartz T and 9 parts of hydroxylamine hydrochloride were dissolved in 100 ml. of methanol.

Table I. Strength of Calcium Chloride Solution

Days Stored	$CaCO_3$ Equivalent[a], Mg./Ml.	Deviation from Average
0	0.992	±0.000
5	0.991	−0.001
30	0.991	−0.001
60	0.992	±0.000
120	0.993	+0.001
	Av. 0.992	0.0006

[a] Average of 5 titrations.

PROCEDURE

The desired amount of calcium chloride solution (30 to 40 ml.) was accurately delivered from a buret into a 250-ml. conical borosilicate glass flask. One milliliter of the buffer solution and 8 to 10 drops of the indicator solution were added, giving a wine-red color. The sodium Versenate solution was added from a buret until the last trace of pink disappeared to give a pure blue.

Each time the sodium Versenate solutions were analyzed, a new standard calcium chloride solution was prepared as indicated above. The secondary standard calcium chloride solution was then standardized against the freshly prepared primary standard calcium chloride solution by titrating aliquots of both solutions with one of the sodium Versenate solutions. All of the sodium Versenate solutions were then checked against the secondary standard calcium chloride solution.

The secondary calcium chloride solution was found to be stable over the 4-month test period (Table I). The stability of the

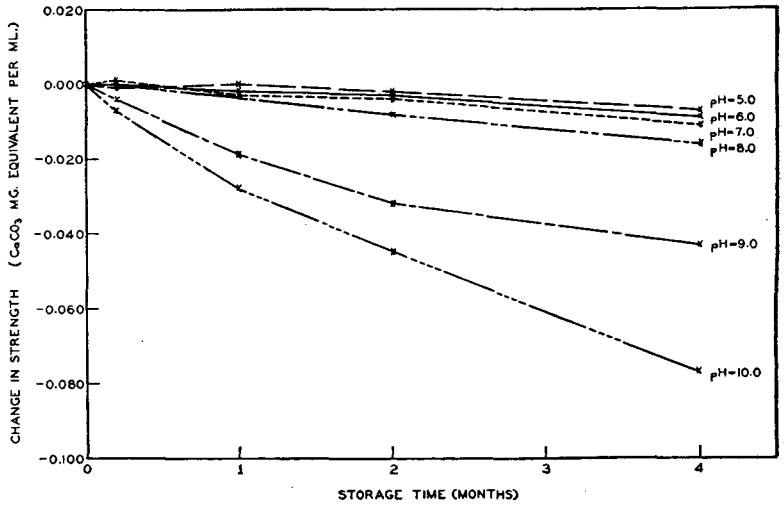

Figure 1. Change in Strength of Versenate Solutions of Varying pH over a 4-Month Period

Strength of solutions about 1 mg. of $CaCO_3$ equivalent per ml.

Table II. Strength of Standard Versenate Solutions of Varying pH over a 4-Month Period

(Given as $CaCO_3$, mg. equivalent per milliliter)

Days Stored	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
0	1.049	1.044	1.062	1.050	1.062	1.047
5	1.048	1.044	1.063	1.050	1.058	1.041
30	1.049	1.042	1.060	1.047	1.043	1.019
60	1.047	1.041	1.058	1.042	1.030	1.002
120	1.042	1.035	1.051	1.034	1.019	0.970

sodium Versenate solutions is shown in Table II and Figure 1. Each datum given is the average of five analyses.

CONCLUSION

It is evident that the lower the pH the more stable the solution. Below a pH of 4.25 there is some tendency for the ethylenediamine tetraacetic acid to precipitate from the solution. A pH of 4.25 to 5 is recommended and is obtained when the analytical grade powdered sodium Versenate is dissolved in distilled water. The data indicate that standard sodium Versenate solutions stored in 1-gallon containers at a pH of 5 will change in strength less than 1% over a 4-month period.

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Determination of the Fluoride Ion with Ferric Thiocyanate

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A study of the ferric thiocyanate complex indicates that the sensitivity of the complex toward the fluoride ion is dependent upon both total ion concentration and concentration of the ferric, thiocyanate, hydrogen, and sulfate ions. The concentrations of the first three of these four ions can be controlled by the amounts of chemicals added in the test, while the concentration of the sulfate ion can be determined roughly by using a second color tube containing the sample and same reagents plus zirconium oxychloride (3.0 mg. in a 100-ml. sample). The increase in the color of the ferric thiocyanate complex brought about by the zirconyl ion is a measure of the fluoride ion concentration. The sensitivity of the complex toward a given amount of fluoride is indicated by the difference between the color intensities of the sample plus zirconyl ions and the color of a tube of distilled water with ferric thiocyanate reagents. The full color intensity of the ferric thiocyanate complex develops instantaneously and is stable in the dark for several hours, though sensitive to blue light. The color does not change in hue, optical density values are reproducible, and sensitivity is similar to the zirconium alizarin technique.

THE quantity of fluoride ions present in potable waters has assumed increasing importance since it was indicated by McCollum and co-workers (*10*) in 1925 that the structure of the teeth of the long-time inhabitants of a community is affected by the fluoride ion concentration in that community's water supply.

In 1936, the American Water Works Association set up a committee to study the various known methods for determining the fluoride ion concentrations of many types of waters. On the basis of this committee's report (*4*), the Standard Methods Committee (*1*) selected the Scott modification (*12*) of the Sanchis method (*11*) which uses a zirconium alizarin reagent that is decolorized by the fluoride ions. The decolorization is estimated by reference to simultaneously prepared standards of known fluoride ion concentrations.

The standard methods technique is simple and as accurate as any of the other techniques that were available at the time it was chosen. It has three disadvantages, however: As the pink or red color formed by the zirconium alizarin lake decreases in intensity with increasing fluoride ion concentration up to a limit of 1.4 p.p.m., the yellow color of the uncombined alizarin dye becomes apparent and a change in hue of the solution results; the rate of color change is large during the first hour, and reproducibility is poor during this period of rapid color change; and the color intensities produced by the fluoride ion standards change with the age of the reagent, even when the same time intervals for color development are allowed.

A search of the literature was made, and laboratory studies showed that the ferron iron method (*7*) for the fluoride ion determination was rapid and not subject to change in hue, but that this technique was not sensitive to concentrations of fluorides less than 1.0 p.p.m. No further work was therefore performed on this method. Work with the thorium nitrate technique (*5*) also indicated that this method was not suitable for a colorimetric procedure.

In 1933, Foster (*8*) described a method in which ferric thiocyanate was used for the determination of fluoride ion concentration. In this method were included necessary corrections for many other ions that might be present in the sample. A study of this procedure indicated that the use of the ferric thiocyanate complex might overcome the three distinct disadvantages of the fluoride ion determination with the zirconium alizarin reagents, for it was found here that the hue does not vary in the analysis, that the color changes are instantaneous, and that the reagents are stable. The ferric thiocyanate technique has been suggested by Chemodanva (*6*) for the measurement of fluoride content of an exhaust air stream, while Babko and Kleiner (*2*) have studied its use for water.

However, as shown in the earlier work (*8*), iron, sulfate ions, and other components of natural waters interfere with the determination of fluoride ions by this method. The effects of these ions have been compensated for, to some extent, in the method described in this paper—the addition of zirconyl chloride to the sample plus reagents partially restores the color intensity of the fluoride ion–containing sample, rather than simply measuring the decolorization of the complex by the fluoride ion as suggested by Foster (*8*). Fluoride ions in a sample of water reduce ferric ion concentration and thus reduce ferric thiocyanate color, while the zirconyl ion reacts more strongly with the fluoride ion than does the ferric ion, so that there is an increase in the intensity of color. The zirconyl ion does not, however, change the sulfate ion concentration or its effect upon the color.

A study was made of the effect of pH, the relative concentrations of the iron and thiocyanate ions, and the effect of the sulfate ion and total ion concentrations on the sensitivity toward 1.0

p.p.m. fluoride ions. The details of the procedure suggested were chosen to provide the most satisfactory general application of the proposed method.

PROCEDURE

Reagent Solutions. Perchloric acid, 1.00 *N;* ferric ammonium sulfate in 0.01 *N* perchloric acid at a concentration of 0.15 mg. of ferric ion per ml.; ammonium thiocyanate stabilized with ammonia to give a solution at pH 7.5 to 8.0 with a concentration of 10 mg. of thiocyanate ion per ml.; and zirconium oxychloride solution at a concentration of 3 mg. of zirconium oxychloride per ml.

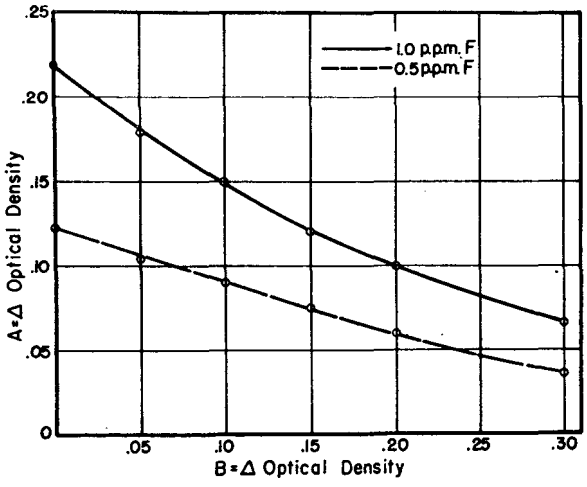

Figure 1. Effect of Interfering Ions upon Sensitivity of Ferric Thiocyanate Complex for Determining Fluoride Ion Concentration

A. **O.D. (sample plus reagents) − O.D. (sample plus reagents plus Zr)**

B. **O.D. (distilled water plus reagents) − O.D. (sample plus reagents plus Zr)**

Apparatus. A photoelectric colorimeter, a glass electrode pH meter, a Lumetron filter photometer No. 450, and a Beckman pH meter Model M were used in this study.

Procedure. In each of two flasks (numbered 1 and 2) are placed 100 ml. of clear sample, 1.0 ml. of 1 *N* perchloric acid is added with mixing, and the pH of the solution is measured. The quantities and concentrations of the reagents are such that the pH of the blank will be 2.0 or 1.9 with the iron and thiocyanate ions and the pH of the samples should be the same value within 0.05 unit (if the pH is not within the limits specified, another aliquot of the sample should be taken and the necessary amount of perchloric acid added). Then 1.0 ml. of the ferric ammonium sulfate solution and 1.0 ml. of the thiocyanate solution are added, the contents of each flask are mixed thoroughly, and 1.0 ml. of the zirconium oxychloride solution is added to flask 1 and 1.0 ml. of distilled water to flask 2. To a third flask (No. 3) containing 101 ml. of distilled water is added 1.0 ml. with mixing of each of the first three reagents (acid, iron, and thiocyanate). The colored samples are transferred to color comparison tubes and the optical density or per cent transmittance is determined relative to water as a reference, using a blue filter with maximum transmittance around 460 to 490 mμ.

The difference in the color density of the first two tubes, *A*, is caused by the fluoride ions, while the difference in color density between the blank (No. 3) and the tube with zirconium (No. 1), *B*, is caused by other ions, primarily sulfate ions. If the sulfate ion and total salt concentrations of the sample are known, the blank (third tube) may not be needed. However, the sensitivity of the ferric thiocyanate complex toward a constant amount of fluoride ions is reduced as the sulfate ion concentration increases until the sensitivity is only 30% of the original at 200 p.p.m. sulfate ions. Thus, by using a chart such as shown in Figure 1 or a nomograph prepared from this chart, as in Figure 2, the fluoride concentration is determined as follows:

The difference between the reading of the first tube (sample plus zirconium ions) and that of the third tube (blank), *B*, locates

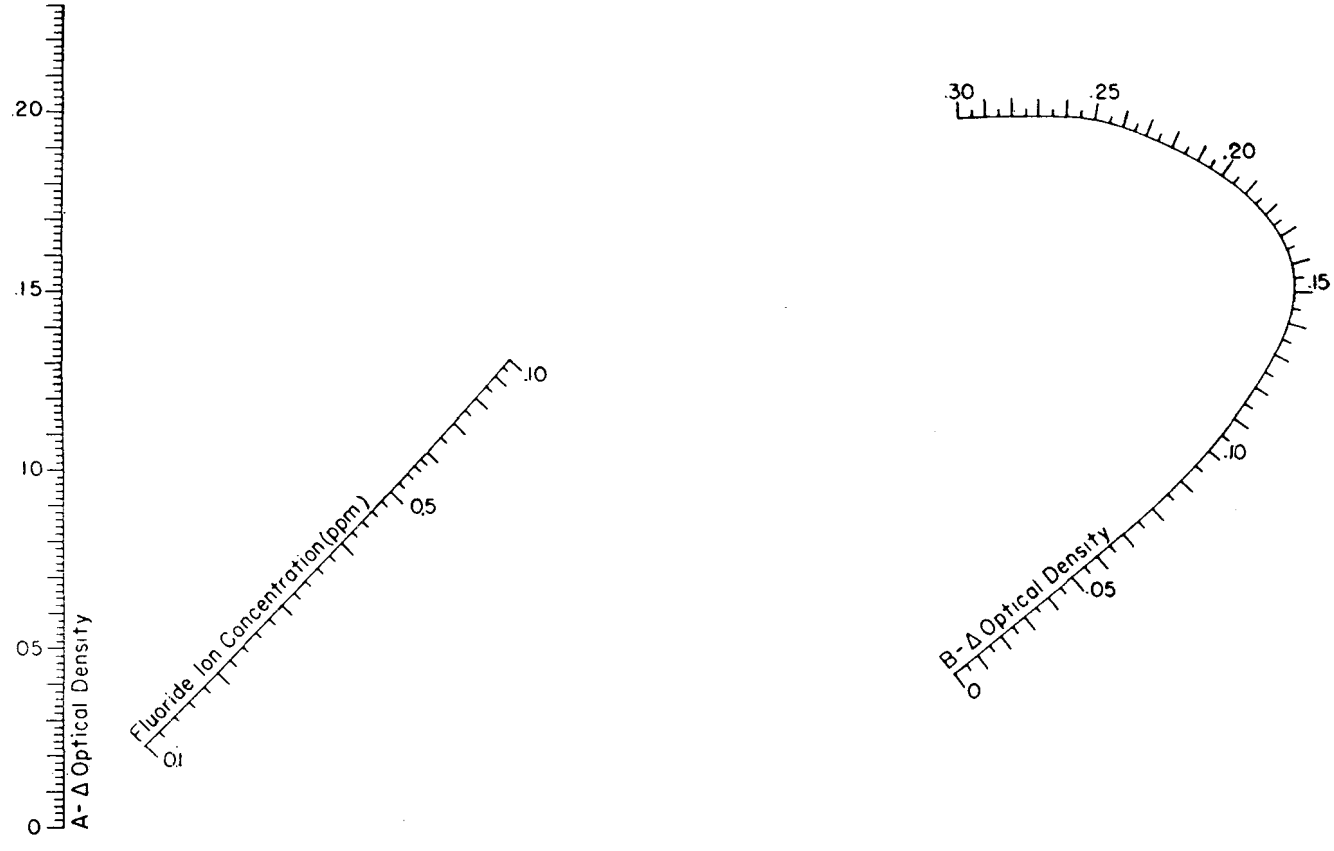

Figure 2. Nomograph for Determination of Fluoride Ions with Ferric Thiocyanate in Presence of Interfering Ions

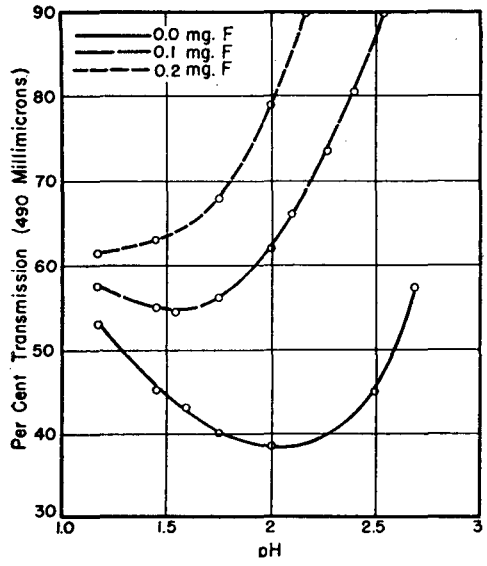

Figure 3. Effect of pH on Color Intensity of Ferric Thiocyanate with and without Fluoride Ions

Table I. Effect of Time upon Color Intensity of Ferric Thiocyanate Complex at 20° C.

Fluoride Ion Concn.	pH	Time after Adding Thiocyanate Ions	Light	Transmittance
P.p.m.		*Min.*		%
0	2.0	1.5	+	45
		11	+	46
		33	+	48
1	2.0	1.5	+	70
		15	+	71
		32	+	72
0	2.0	240	Absent	45
		960	Absent	46

the portion of the fluoride chart to use (the abscissa of Figure 1), while the difference in the readings between the first two tubes, A, indicates the quantity of fluoride ions (the ordinate of Figure 1).

This method is particularly adaptable to the determination of an increased fluoride ion concentration where there is no change in the concentration of interfering ions during the addition of the fluoride ions as indicated by Foster (*8*).

EXPERIMENTAL RESULTS

Color Spectrum of Ferric Thiocyanate Complex. It was found that the greatest sensitivity could be obtained by using a filter which had a maximum transmittance at 490 mμ, as recommended by Bent and French (*3*).

Stability of Color of Ferric Thiocyanate Complex. Because one of the principal difficulties of the zirconium alizarin technique is the rapid initial change in color, a possible substitute method must have a color that is instantaneous and stable in its formation. The results shown in Table I, which deals with the effect of time on the color intensity of the ferric thiocyanate complex at 20° C., indicate that the color changes of the ferric thiocyanate color complex occur within 90 seconds and that there is a slow destruction in color intensity of the sample when it is exposed to the light, but that no destruction in color intensity occurs in the dark or in red light for at least 4 hours.

Optimum pH and Concentration of Reactants. Chemically, the intensity of color in a solution of the ferric thiocyanate complex depends on the extent of the successful competition of the thiocyanate ions with the interfering fluoride, hydroxide, sulfate, chloride, nitrate, and perchlorate ions for the ferric ions present. The hydroxide, sulfate, and fluoride ions, in particular, combine chemically with the ferric ions to displace the thiocyanate ions, as may be noted from the data plotted in Figure 3. It is evident from the lowest line of this figure that as the hydroxide ion concentration decreases, the thiocyanate ions can form more of the colored complex until the pH reaches 1.9 to 2.0.

A further decrease in pH brings about a decrease in color intensity, caused by two factors: (1) At pH values less than 1.9 the ionization of the thiocyanic acid ($Ki = 10^{-3}$) is decreased by the increased hydrogen ion concentrations, and (2) the higher ion concentration of the extra acid needed to lower the pH causes interference in the colored ferric thiocyanate complex formation by decreasing the activity coefficients of the reactants. At pH 1.2 it can be shown that approximately two thirds of the color decrease is caused by the decreased thiocyanate ion concentration, while the remaining one third is caused by the larger numbers of ions in the solution.

The upper two lines of Figure 3 represent results obtained by adding fluoride ions to the ferric thiocyanate complex at various pH values. It is evident from this figure that the fluoride ions form a tighter bond with ferric ions than do the thiocyanate ions.

From these data, it can be shown that the pH (1.9), selected for this procedure, is not the point of greatest sensitivity of the ferric thiocyanate complex toward 1.0 p.p.m. fluoride ions, but that the greatest sensitivity actually occurs at pH 2.5. However, pH 1.9 was chosen instead of pH 2.5 in order to give a slightly greater range of fluoride ion concentrations than is possible at pH 2.5 and to reduce the requirements for the accuracy with which the pH must be adjusted for necessary reproducibility.

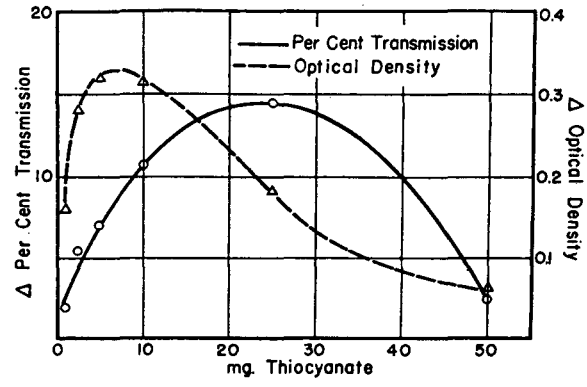

Figure 4. Effect of Changes in Thiocyanate Ion Concentrations

On sensitivity toward 1.0 p.p.m. fluoride ions by ferric thiocyanate at constant pH and 1.5 p.p.m. ferric ion

In choosing the optimum ferric and thiocyanate ion concentrations for the determination of 1.0 p.p.m. fluoride ions, many hundreds of determinations were made. The problem is complicated by the variable effect of pH and a desire to employ the sensitive portion of the range of colorimetric instrument. In reporting the results of this part of the study, the data are presented in both optical density and per cent transmittance in order to facilitate the application to instruments other than the Lumetron No. 450. In order to measure the concentration of a dissolved solute with the smallest percentage error, solutions should be used with optical densities between 0.3 and 0.6 (25 to 70% transmittance). However, for a given cell length, the largest changes in scale reading upon the addition of fluoride ions for a given concentration of the reactants may not occur in the most sensitive range of the instrument. Figures 4, 5, and 6 present data which allow the most satisfactory choice of reactant concentrations to be made for any particular instrument and cell length.

Results shown in Figure 4 indicate that at 1.5 p.p.m. ferric ion concentration the thiocyanate ion concentration is very important in determining the sensitivity of the ferric thiocyanate com-

plex toward 1.0 p.p.m. fluoride ions (at pH 1.9). From the curve representing changes in per cent transmittance, it can be seen that with 0.15 mg. of ferric ions the 10 mg. of thiocyanate ions recommended in this procedure give the maximum sensitivity for this instrument, but that 25 mg. of thiocyanate ions would give a greater change in optical density (color). However, this change could not be read on the Lumetron photometer as accurately as the change at 10 mg. of thiocyanate ions.

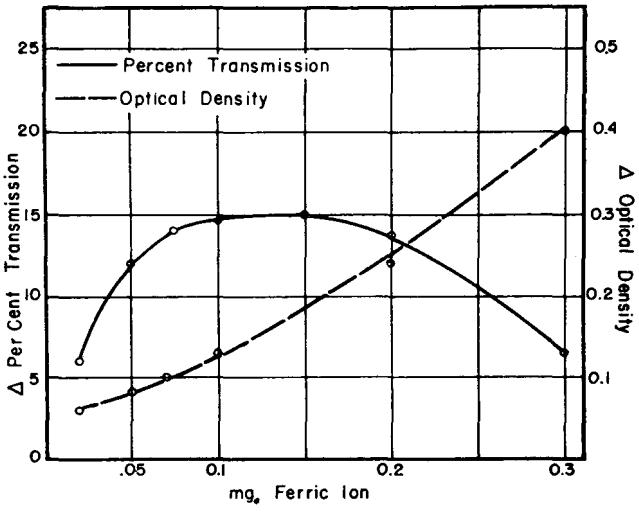

Figure 5. Effect of Changes in Ferric Ion Concentrations

On sensitivity toward 1.0 p.p.m. fluoride ions caused by ferric thiocyanate at constant pH and 100 p.p.m. thiocyanate ions

Before discussing this choice further, the results with varying ferric ion concentration with constant thiocyanate ion concentration should be considered. These results, shown in Figure 5, indicate that the optimum change in per cent transmittance also occurs at the recommended ferric ion concentration (0.15 mg. of iron). However, the largest change in optical density occurs with a concentration of ferric ions greater than that recommended, but again it occurs at an optical density larger than can be determined with the necessary accuracy when the instrument uses the 150-mm. light path.

It was necessary to know whether this increase in the color change at much higher optical densities could be used to advantage in a shorter light path. This question was answered by studying the ratio of the initial color intensity to the change in color intensity produced by the addition of 1.0 p.p.m. fluoride ions. Because higher ferric or thiocyanate ion concentrations cause higher initial color intensities, it was necessary to use light paths of 75 and 37.5 mm. for this study. Several selected ferric ion concentrations were chosen, and a range of thiocyanate ion concentrations was used at each ferric ion concentration. Curves were plotted of the change in optical density caused by 1 p.p.m. fluoride ions against the initial optical density of the reagents alone. These procedures were repeated at different ferric ion concentrations for several thiocyanate ion concentrations, and a second family of curves was plotted. The two families of curves have been simplified by choosing a fixed initial optical density of 0.45 and plotting the change in optical density produced by 1.0 p.p.m. fluoride ions, first, against the thiocyanate ion concentration required to produce the optical density of 0.45, and then against the ferric ion concentration required to produce the optical density of 0.45. These curves are shown in Figure 6. The optical density of 0.45 was chosen because it is within the range of maximum accuracy of the instrument that would include data from the low ferric and thiocyanate ion concentrations.

The data of Figure 6 indicate that the thiocyanate ion concentration should be at least 40 p.p.m. (4 mg.) for determining 1 p.p.m. fluoride ions at the ferric ion concentration required to produce an initial optical density of 0.45. As the thiocyanate ion concentration is increased beyond 40 p.p.m., the 1 p.p.m. fluoride ions have more trouble competing with the thiocyanate ions for the ferric ions. On the other hand, there is a broad range of ferric ion concentrations over which there is very little change in sensitivity, but a minimum ferric ion concentration appears to be absolutely necessary. These data would indicate that for a shorter light path than 150 mm. a concentration of 40 p.p.m. thiocyanate ions should be chosen along with the amount of ferric ions required to obtain an optical density of 0.45 to 0.6 in 101 ml. of distilled water adjusted to pH 2.0 with perchloric acid. The higher optical density of 0.6 requires 100 p.p.m. thiocyanate ions at 1.5 p.p.m. ferric ions for maximum sensitivity against 1.0 p.p.m. fluoride ions, as may be determined when the data are developed in a manner similar to that of Figure 6. When it is essential to be able to determine more than 1.0 p.p.m. fluoride ions, more than 40 p.p.m. thiocyanate ions will be required as the necessary minimum. Thus, at least 100 p.p.m. (10 mg.) thiocyanate ions are considered to be nearly the optimum value.

Effect of Sulfate Ion Concentration. Sulfate ion concentration is a fifth and important variable because of the previously mentioned competition between the sulfate and fluoride ions. Foster (*8*) recognized this problem and recommended the addition of compensating quantities of ferric ions for those removed by the ferric sulfate complex. Data in Table II indicate that loss in color caused by sulfate ions may be restored by the addition of more ferric or thiocyanate ions, but that the sensitivity of the complex toward 1.0 p.p.m. fluoride ions is not fully restored in the presence of the extra ferric or thiocyanate ions because of the decrease in activity coefficients of the ferric and thiocyanate ions. Thus, the addition of extra ferric or thiocyanate ions is not recommended here, but the effect of the sulfate and the total concentration of other ions discussed earlier is instead determined directly by the use of zirconyl chloride.

Representative Analyses by This Technique. Table III shows the results of the analyses of a number of natural water samples

Table II. Effect of Extra Ferric or Thiocyanate Ions on Sensitivity of Ferric Thiocyanate Complex toward 1.0 P.P.M. Fluoride Ions in Presence of 100 P.P.M. Sulfate

Quantity of Each Ion per Tube					Optical Density	
Ferric *Mg.*	Thiocyanate *Mg.*	Sulfate *Mg.*	Fluoride *Mg.*	pH	Reading	Change by fluoride ions
0.15	10	..	...	2.0	0.60	
0.15	10	..	0.1	2.0	0.39	0.21
0.15	10	10	...	2.0	0.41	
0.15	10	10	0.1	2.0	0.31	0.10
0.215	10	10	...	2.0	0.58	
0.215	10	10	0.1	2.0	0.43	0.15
0.15	16.5	10	...	2.0	0.59	
0.15	16.5	10	0.1	2.0	0.44	0.15

Table III. Fluoride Ion Content of Samples of Water[a], P.P.M.

	Zirconium Alizarin			Ferric Thiocyanate		
Sample	Direct	0.1 mg. F^- added to 100 ml.	Difference[b]	Direct	0.1 mg. F^- added to 100 ml.	Difference[b]
1	0.1	0.96	0.0	0.1	1.1	0.1
2	0.4	1.00	0.0	0.1	1.2	0.2
3	0.2	1.00	0.0	0.1	1.1	0.1
4	0.3	1.2	0.2	0.1	1.0	0.0
5	0.0	1.0	0.0	0.2	1.2	0.2
6	0.1	1.1	0.1	0.2	1.2	0.2
7	0.1	1.1	0.1	0.1	1.1	0.1
8	0.1	1.1	0.1	0.0	1.0	0.0
9	0.1	1.1	0.1	0.1	1.1	0.1
10	1.5	2.5	1.5	1.6	2.6	1.6
11	0.2	1.2	0.2	0.4	1.4	0.4
12	0.4	1.4	0.4	0.4	1.4	0.4

[a] Determinations of other ions are given in Table IV.
[b] Amount indicated as present originally, obtained by subtracting amount added from amount recovered in columns 2 and 5.

Table IV. Determination of Common Mineral Ions in Water Samples in P.P.M.

Sample	Ca^{+++}	Mg^{++}	Fe^{++}	NH_3 N	NO_3 N	NO_2 N	Cl^-	SO_4^{--}	PO_4^{--}	SiO_2^-	CO_2	pH	$CaCO_3$ Alkalinity	$CaCO_3$ Hardness
1	62	6	0.00	0	3	0.02	98	57	<0.05	28	30	6.8	90	182
2	30	1	0.00	0.8	30	0.06	16	0	<0.05	27	4	7.0	18	64
3	56	6	0.00	0.9	6	0.06	137	45	0.06	10	20	6.6	57	164
4	9	1	0.02	0.9	1.2	0.08	0	<1	0.12	11	11	6.7	35	22
5	5	3	0.10	1.0	0.2	0.07	0	0	0.08	9	11	6.7	33	24
6	12	1	0.20	1.0	3	0.07	0	0	0.00	3	3	7.3	36	31
7	32	1	0.00	0.0	5	0.08	12	18	0.00	9	15	6.8	59	82
8	23	<1	0.00	0.0	5	0.08	11	0	<0.10	8	25	6.6	49	62
9	9	<1	0.16	0.0	0.8	0.08	0	0	0.00	8	29	6.3	33	20
10	75	17	0.00	0.0	11	0.05	5	39	<0.05	5	135	6.5	228	260
11	23	4	0.02	0.0	0.2	0.06	3	9	<0.10	10	4	7.4	66	74
12	23	6	0.05	0.0	0.2	0.07	21	22	0.10	11	13	7.6	47	84

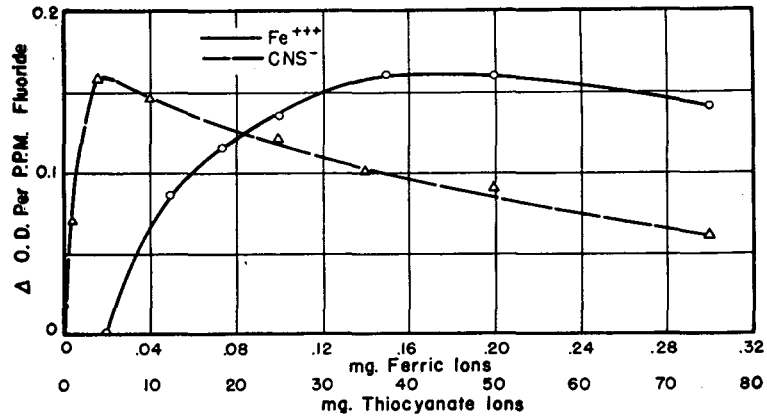

Figure 6. Sensitivity of Ferric Thiocyanate

Toward 0.1 mg. fluoride ions in 100 ml. (1.0 p.p.m.) at optical density of 0.45 with different ferric and thiocyanate ion concentrations

for fluoride ions by this method and by that of standard methods (*1*). Also included are data which show the effect of adding more fluoride ions to each of the samples when the sample plus the increment in fluoride ions is analyzed by each method. The values for the fluoride ion concentrations by the ferric thiocyanate test are in good agreement with the values indicated by the addition of more fluoride ions in both this test and the zirconium alizarin method. It is realized that the results cover a limited range of concentration, but it is not necessary to check concentrations above 1.4 p.p.m. of fluoride ions when the standard methods technique is concerned, inasmuch as this is the limit of that test.

A rather complete mineral analysis for each of the waters is given in Table IV.

DISCUSSION

Although the proposed method requires the use of both a pH meter and a colorimeter and has five variables, so that it is somewhat complicated, it is sensitive, rapid, and based upon the change in intensity of color of a single hue. It should prove particularly valuable in controlling the amount of sodium fluoride added to the drinking water in communities such as Grand Rapids, Mich. (*9*). The color of the ferric thiocyanate is stable in the dark or in red light, and the changes caused by sulfate or fluoride ions are apparently instantaneous and reversible. Thus, an immediate, positive control of any fluoride ion addition is possible, making use of the decrease in color of the ferric thiocyanate complex.

In the analysis of an unknown water sample, one can use either the results of the analysis for the sulfate and other ions in choosing the sensitivity range, or simply make up a "blank" tube and obtain a rough sulfate ion value by this new technique while determining the fluoride ion concentration. Thus, *B* (the abscissa of Figure 1, difference between the blank and the sample with zirconium ion) may be plotted against a known sulfate ion concentration and the sulfate ion value of an unknown sample read from the curve. When this was done during the complete mineral analyses of the samples shown in Tables III and IV, good correlation in many of the sulfate ion values was obtained both by this colorimetric procedure and by the volumetric procedure with benzidine hydrochloride (as modified in the authors' laboratory). Thus, several samples showed no sulfate ions by either technique, whereas one sample showed 34 p.p.m. colorimetrically and 39 p.p.m. volumetrically. The authors recommend the use of the colorimetric determination of sulfate ions with this fluoride ion test because a high chloride or nitrate ion concentration gives an appreciably higher apparent sulfate ion value than is obtained volumetrically.

If a greater range in the fluoride ion determination than is given at pH 1.9 is needed, standard curves at pH 1.7 can be set up, for this would give greater range at reduced sensitivity. It is realized that the sensitivity is not a maximum at pH 1.9, but reproducibility in the color intensity of the blank is better at pH 1.9 than at pH 2.3 to 2.5 where there is a larger relative change in color of the ferric thiocyanate complex with change in pH, in both the absence and presence of the fluoride. This is caused by the highly competitive nature of the hydroxyl ion at these pH values.

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Electrolytic Reductions at Constant Cathode Potentials

Electronically Controlled Apparatus

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An electronically controlled apparatus is described for electrolytic reduction at constant cathode potentials. This instrument is extremely sensitive and stable with negligible "hunt" characteristics. It is applicable to currents as high as 20 amperes and 75 volts applied voltage. With the plot of current against time it is possible to determine the total current consumed in the electrolytic reduction. The instrument does not require preliminary calibration and can be used to give a plot of cathode potential against current of solutions too concentrated to be polarographed with the standard instruments.

IN THE course of research it became necessary to develop an instrument for the electrolytic reduction of organic compounds at constant cathode potentials. In addition, the instrument had to be extremely responsive to changes in the amount of reducible substance in the catholyte and be capable of delivering the relatively high currents or applied voltages called for by high cathode potentials. It also had to be sensitive and responsive enough to give voltammetric curves (*9*) of solutions of such concentrations of reducible substance which could not be handled by the polarograph.

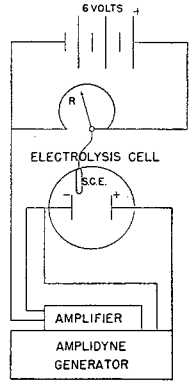

Figure 1

Haber in his classical work on the reduction of nitrobenzene at constant cathode potential (*6*, *7*) used an instrument in which the total applied voltage was manually adjusted so as to maintain a constant cathode potential. Hickling (*8*) developed an all-electronic unit which unfortunately could deliver only small currents. Although the apparatus designed by Caldwell, Parker, and Diehl (*5*) could deliver higher currents, the total applied voltage obtainable was in the neighborhood of 10 volts. Lingane's apparatus (*10*), although automatic, had certain disadvantages for the author's work. This unit as described could deliver only 10 to 15 volts and a maximum current of about 4 amperes with good conductors. Although his instrument had a sensitivity of ±0.02 volt, its response was somewhat slow, as would be expected in such a mechanical device.

The instrument described here utilizes an electronic amplidyne as a control and current source. It gives the relatively high currents or applied voltages required for the reduction of many organic compounds at cathode potentials as high as 6 volts and will control in both directions. Its sensitivity is ±0.005 volt. The parts used in the construction of this instrument are readily available and easily assembled and can be built for approximately $1000.

Essentially this instrument will give automatically controlled impressed potential to secure constant cathode potential. The desired cathode potential is obtained by opposing the e.m.f. of the standard calomel electrode with an e.m.f. from potentiometer *R*, as shown in Figure 1.

During an electrolysis experiment as the amount of reducible substance diminishes, the e.m.f. between the calomel cell and the opposing e.m.f. from the potentiometer will become unbalanced. This condition will transmit itself to a bank of tubes in the amplifier, causing the amplidyne generator to change the total applied voltage to the electrolysis cell in order to establish a balanced condition again between the standard calomel electrode e.m.f. and the potentiometer e.m.f. As the electrolysis proceeds and the amount of reducible substance diminishes, the current will automatically drop and reach a plateau when the electrolysis is completed.

As can be seen from Figure 2, which is a typical curve of current *vs.* time obtained as a result of a bimolecular reduction of *p*-hydroxybenzaldehyde to 4,4′-dihydroxyhydrobenzoin (*1*), the degree of hunt is negligible and as the reduction proceeds the current drops until a plateau is reached, indicating completion of the reaction. Each vertical space on the chart represents 1.93 minutes and each horizontal space represents 0.05 ampere.

Figure 3 gives the schematic details of the assembly.

The total applied voltage and current delivered to the electrolysis cell are indicated by voltmeter *V*2 (Weston Model 643 D.C. panel voltmeter, 0- to 100-volt scale) and ammeter *A* (Weston Model 931 multiple-range ammeter indicating 0 to 2.5, 0 to 10, and 0 to 25 amperes). A three-point rotary switch, *S*5, controls the ammeter range.

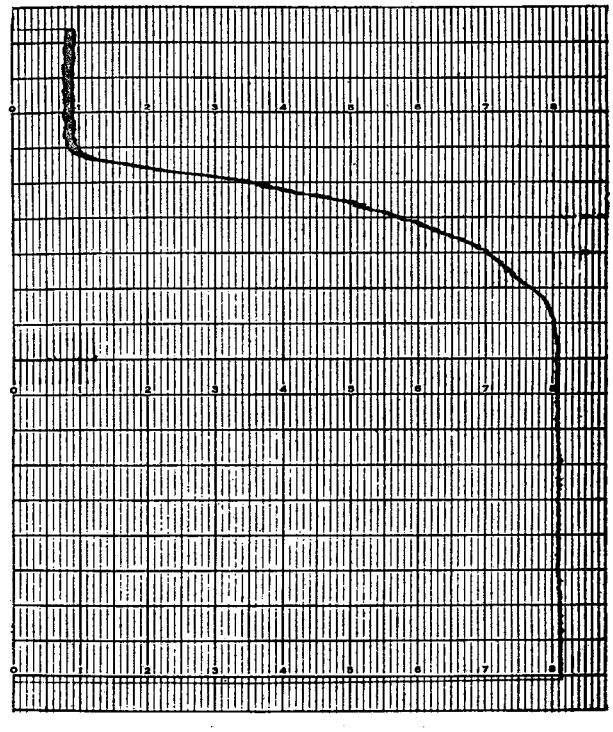

Figure 2. Current *vs.* Time

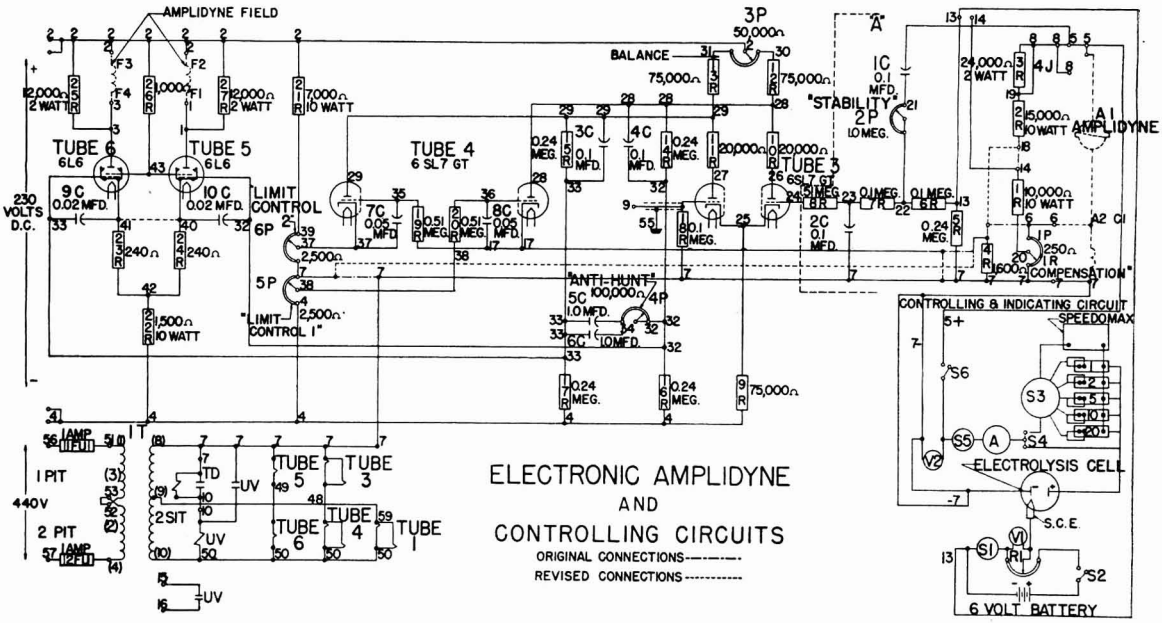

Figure 3

A plot of current *vs.* time is obtained with a Speedomax recorder (Leeds & Northrup No. 60101-S Model S Speedomax potentiometer-type indicating recorder) which has a scale of 0 to 100 divisions and a range of 0 to 50 mv. The external circuit consists of five 50-mv. shunts (Weston portable testing shunts) of values 1, 2, 5, 10, and 20 amperes, which gives the recorder variable ranges of 0 to 1, 0 to 2, 0 to 5, 0 to 10, and 0 to 20 amperes. The variability of the Speedomax range is controlled by *S*3, a five-point rotary selector switch (Leeds & Northrup Model 8240). The recorder can be placed in or out of the circuit by *S*4, a three-position toggle switch, the center position of which prevents the current from flowing through the electrolysis circuit.

The desired cathode potential is obtained by placing the standard calomel electrode against the surface of the cathode. This e.m.f. is opposed by an e.m.f. from a 100-ohm radio-type potentiometer, *R*1 (General Radio Company, Type 301), which is powered by four 1.5-volt dry cells connected in series. The cathode potential is read on voltmeter *V*1 (Weston Model 931 multiple-range voltmeter indicating 0 to 1.5, 0 to 3, and 0 to 6 volts). A three-point rotary switch, *S*1, controls the range of this voltmeter. A toggle switch, *S*2, opens or closes this circuit.

The off balance between the e.m.f. of the cathode reference electrode and the opposing e.m.f. of *R*1 sends a signal through wires 7 and 13 to an electronic amplifier which is the controlling unit for the amplidyne generator (General Electric Model AM79AB290 wound for 75 volts and 20 amperes for 2 hours or 12 amperes continuous). This off-balance signal causes the amplifier to adjust the output of the generator through wires 5 and 7 in order to correct the unbalanced condition. The amplifier as obtained from the General Electric Company must be revised as shown in Figure 3, in order to control at a constant cathode potential. Toggle switch *S*6 opens or closes the circuit from the generator.

In this circuit the response is instantaneous with negligible "hunt" and with excellent stability over the complete voltage range.

Figure 4 shows the completely assembled unit.

To perform an electrolytic reduction the generator and amplifier are turned on and allowed to warm up for about 1 minute. Switch *S*6 is closed and switch *S*4, a three-position toggle switch, the center position of which will give an open circuit, is closed in either of the two closed positions. One of these closed positions will cause the current to flow through the Speedomax with the resultant plot of current against time. From the area under this curve it is possible to compute the total current used. This has been found to agree with the values obtained using a silver coulometer. The other closed position will cut the Speedomax out of the circuit if so desired. Rotary switches *S*1, *S*3, and *S*5 are adjusted to the desired meter ranges. Switch *S*2 is closed

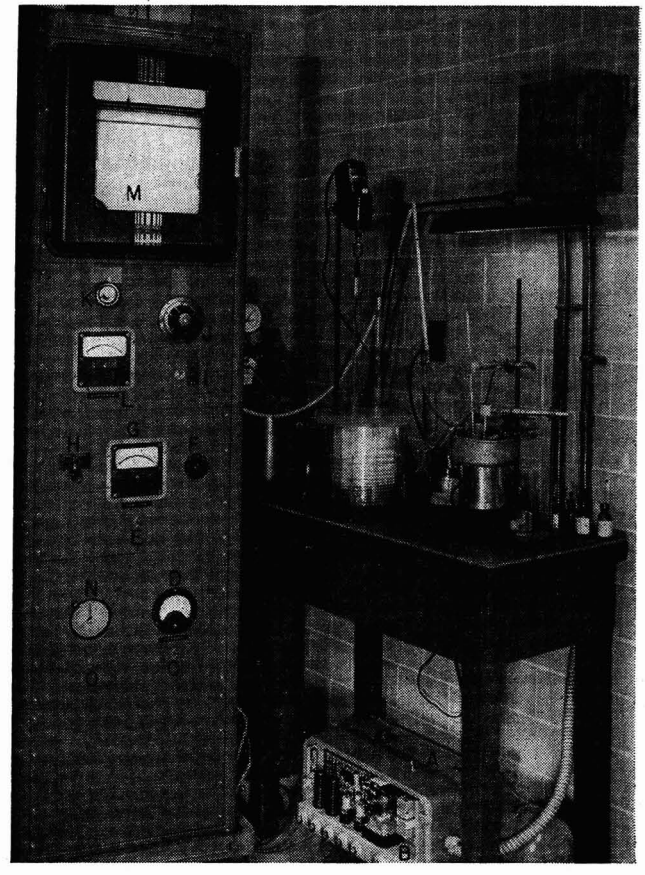

Figure 4. Assembled Unit

A. Amplidyne generator
B. Electronic amplifier
C. Toggle switch *S*6
D. Applied voltage meter *V*2
E. Toggle switch *S*2
F. Radio-type potentiometer *R*1
G. Cathode potential voltmeter *V*1
H. Voltmeter range selector *S*1
I. Three-position toggle switch *S*4
J. Rotary selector *S*3 for shunts to Speedomax
K. Range selector switch *S*5 for electrolysis current ammeter
L. Electrolysis current ammeter
M. Speedomax recorder
N. Timer reading to 0.1 minute and totaling 1000 minutes (Standard Electric Time Company, Model S-600)

and the desired cathode potential with respect to the standard calomel electrode is obtained by rotating potentiometer $R1$.

When a plot of cathode potential against current is desired, the instrument is started in the same manner. $R1$ is rotated to give the desired cathode potential increments and the respective currents are indicated on either the Speedomax or ammeter.

The instrument has been used successfully for the preparation of the pinacols of *p*-aminoacetophenone (*3*) and *p*-aminopropiophenone (*4*). Attempts to prepare these compounds by the usual chemical means resulted in either a negligible yield or no yield at all, whereas by electrolytic reduction large yields were obtained.

In the preparation of other pinacols—i.e., acetophenone pinacol, *p*-methoxyacetophenone pinacol—from their ketones the yields were also higher.

Reductions were also performed on *p*-dimethylaminobenzaldehyde (*2*) and *p*-hydroxybenzaldehyde (*1*) with resultant high yields of the respective hydrobenzoins.

It has also been possible to prepare many amines from their nitro compounds and convert ketones to alcohols.

In general, electrolytic reduction resulted in better yields and cleaner products. The time consumed in the preparation is much less than that ordinarily required using chemical methods.

It is anticipated that this instrument will find wider use as work progresses.

An apparatus of this type can be obtained from the American Instrument Company, Silver Spring, Md.

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Determination of Monomer in Partially Polymerized Acrylic and Allyl Esters

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The partially polymerized mixture is precipitated in a very finely divided state from a pyridine solution by dropwise addition to aqueous bromide-bromate reagent. The bromine released upon acidification adds quantitatively to the finely dispersed sample and the excess bromine is determined by conventional iodometric analysis. This analytical procedure was developed primarily to follow the course of bulk polymerization reactions.

A SUITABLE procedure for the determination of unsaturation by halogen addition to partially polymerized acrylic ester has not been found in the literature. The difficulty in this type of analysis is partly due to the reluctance of halogens to add to a double bond conjugated with an acid group (*2*, *3*), as in the case of acrylic acid, CH_2=CH—COOH, and partly to the problem of exposing the unreacted bonds in the mixture to the action of the addition reagent.

No satisfactory method of adding halogen to a solution of the partially polymerized acrylic esters in organic solvents was found. It has been reported by Robertson and his co-workers (*10*) that hydrogen bromide catalyzes the addition of bromine to acrylic acid in nonaqueous solvents, but the rate was still too slow to make this reagent practical. Although Cohen and co-workers (*4*) reported that bromine added quantitatively to partially polymerized acrylic esters in acetic acid, the authors could not duplicate their results. Their analysis of polyallyl ester mixtures was found to be unsatisfactory. Wijs solution, prepared according to Kemp and Mueller (*7*), was found to be ineffective on solutions of the acrylic polymer mixture in acetic acid and carbon tetrachloride, although it gave satisfactory results with allyl mixtures. An attempt was made to prepare a stable, active bromine reagent by forming bromine salts with pyridine, ethanolamine, and other organic amines in organic solvents, but these reagents proved to be unstable. Sluggish reactions were obtained using bromide-bromate reagent in methanol, with just enough water to keep the bromine in solution.

Aqueous bromide-bromate solution has been shown to be a strong halogenating reagent (*9*). Mercury, aluminum, and nickel salts (*5*) were reported to promote bromine addition (*8*) in some of the more difficult cases; however, the authors have found these salts to be without appreciable effect on acrylic esters. When an attempt was made to form the soluble sodium salts of acrylic esters according to the hydrolytic procedure of Bartlett and Altschul (*1*) the monomers partially polymerized and gave low analyses. Their procedure was found satisfactory for the allyl esters and might be useful to supplement the authors' procedure for highly polymerized polyallyl esters which are insoluble in pyridine. From the work of Evans and Tyrrall (*6*) it was inferred that bromide-bromate reagent adds quantitatively to emulsions of polyacrylates. When various solvents were tested, pyridine was found to have the properties requisite for the production of the polymer mixture in a state of division suitable for this type of analysis: good solubilization of the polyester, water solubility, a

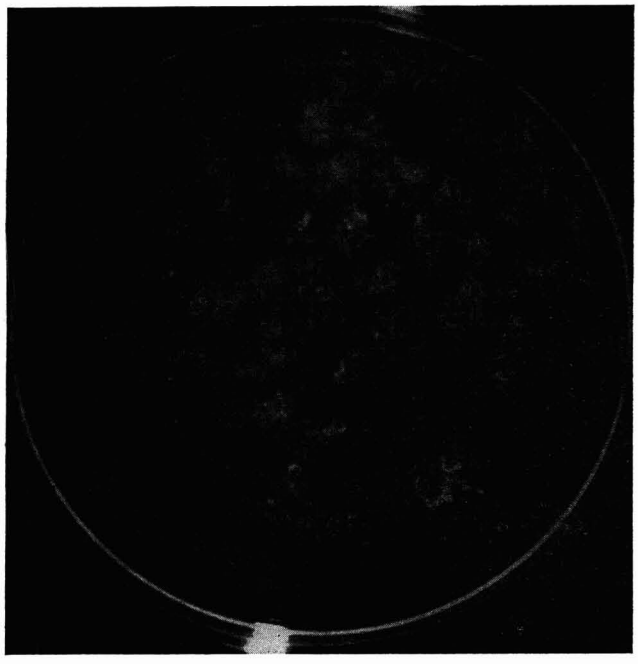

Figure 1. Methyl Methacrylate Polymer

density less than 1, and stability toward bromine in acid solution. A quantitative saturation of partially polymerized material precipitated from pyridine was obtained with aqueous bromide-bromate reagent.

ANALYTICAL PROCEDURE

An accurately weighed sample (0.04 to 0.50 gram) of the partially polymerized acrylic ester is dissolved in and made up with pyridine to a volume of exactly 25 ml. A 5-ml. aliquot of the pyridine solution is added dropwise from a pipet to 20 ml. of a 0.025 *N* to 0.05 *N* bromide-bromate solution in a 250-ml. glass-stoppered Erlenmeyer flask. An effort must be made to distribute the drops evenly over the surface of the brominating reagent in order to secure the best possible dispersion of the polymer (see precipitate in Figure 1). The solution is acidified with 70 ml. of 10% sulfuric acid, stoppered, swirled, and allowed to stand in the dark for 20 minutes at 20° to 25° C. Ten milliliters of carbon tetrachloride are then added and the flask is shaken in order to dissolve the solid polymer. One milliliter of 30% potassium iodide solution is added and the free iodine liberated by the excess bromine is immediately titrated with 0.05 *N* sodium thiosulfate solution, a few drops of fresh starch solution being used as an indicator.

The procedure may be used for determining unsaturation of partially polymerized allyl esters if the addition of bromine is carried out at 0° C. and the sample is allowed to stand for only 10 to 15 minutes. This temperature-time change is necessary to avoid bromine substitution, because the allyl esters are much more active in this respect than are the acrylates.

CALCULATION

The percentage of unsaturation in the polymer mixture is calculated as monomer according to the equation:

$$S = \frac{(A - B) \times M \times 50}{W \times 0.2E} \qquad (1)$$

where S is the per cent monomer, A is the equivalents of bromine, B is the equivalents of thiosulfate, M is the molecular weight, W is the sample weight, and E is the number of double bonds in the monomer.

DISCUSSION

This procedure is applicable only to polymers that are soluble in pyridine. This includes all the polymers formed from monofunctional monomers and the low molecular weight linear polymers formed from polyfunctional monomers.

The sample weight is critical. The weight of liquid samples must not exceed about 0.5 gram; otherwise insufficient dispersion of the sample will prevent quantitative bromination. The critical weight for solid samples is about 0.04 gram. Sample size between these two extremes should be altered proportionally.

Figure 1 is a photograph of methyl methacrylate polymer precipitated by adding 5 ml. of pyridine-polymer solution dropwise to 20 ml. of water in a Petri dish. Most of the precipitate is so finely divided that it appears as a white mist. Figure 2 shows the contents of the dish shown in Figure 1 after stirring. The white

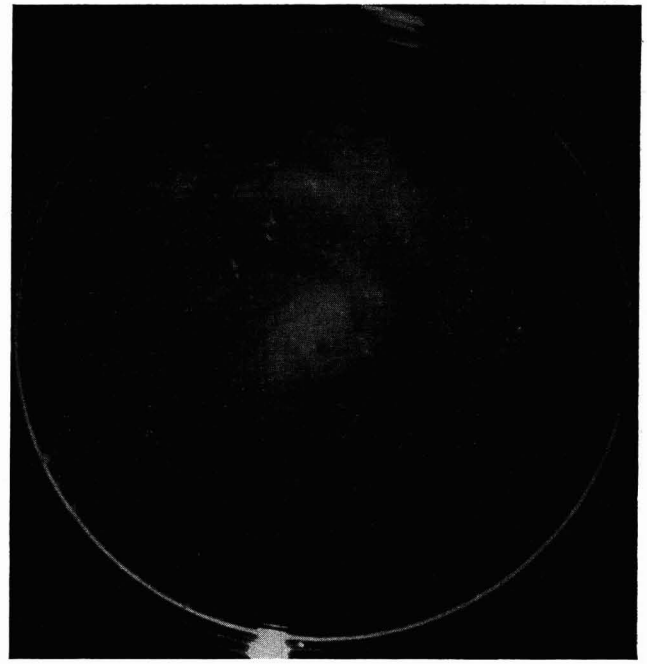

Figure 2. Polymer of Figure 1 after Stirring

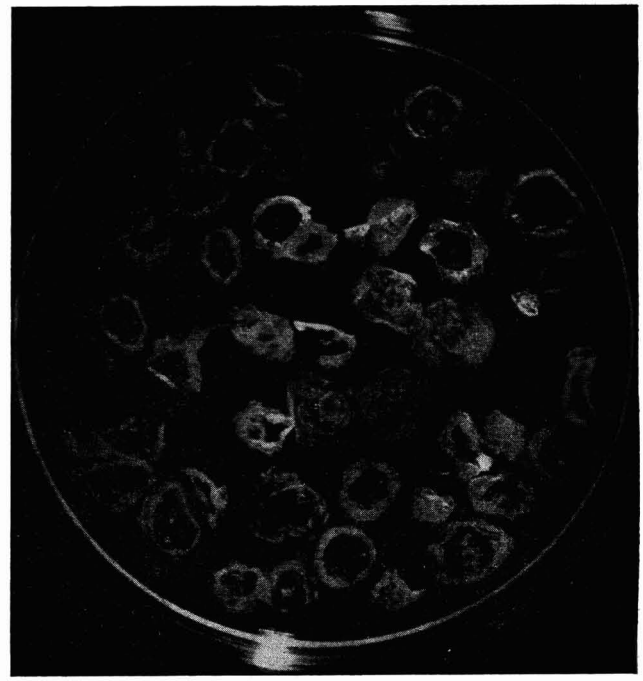

Figure 3. Polymer Precipitated from Acetic Acid Solution

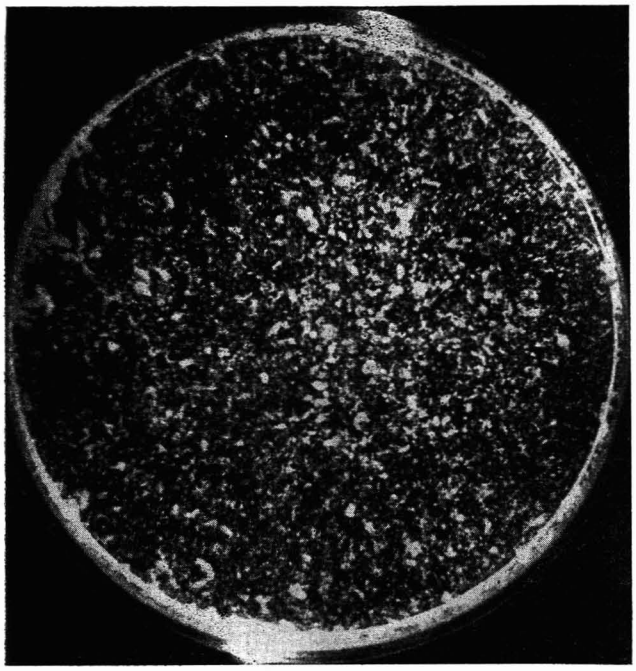

Figure 4. Polymer of Figure 3 after Stirring

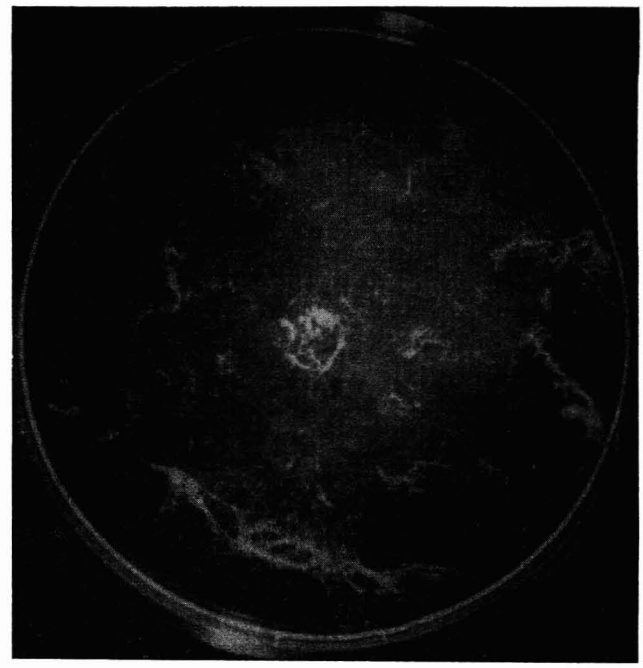

Figure 5. Polymer Precipitate from Too Concentrated Pyridine Solution

mistlike precipitate does not tend to coalesce, but remains in a finely divided state. These particles were seen to be in violent Brownian movement when observed under the microscope. The small white polymer aggregates seen in Figures 1 and 2 were found to be very fragile and porous and not to interfere with the analysis. Figure 5 represents the polymer precipitate from a pyridine solution which was too concentrated. The large gummy polymer aggregates that are formed tend to stick together and do not add bromine quantitatively. This condition must be avoided. Figure 3 shows the hard, dense, fragile polymer precipitated from acetic acid solution, and Figure 4 shows the effect of stirring this precipitate. It is obvious that acetic acid is not a suitable solvent for dispersing the polymer.

It is necessary to use about 100% excess bromide-bromate reagent with acrylic esters in order to force the reaction to completion in the allotted time. Samples allowed to stand for 1.5 hours did not react with more bromine than those which stood only 20 minutes; hence, it is felt that the addition reaction was complete and that no appreciable substitution occurred. As much as 100% excess bromine may be used with allyl esters. The time and temperature specified must be adhered to closely, however, in order to avoid appreciable substitution of bromine. Because the allyl bond is more reactive than the acrylic bond, it may be halogenated simply by dissolving the sample in a suitable organic solvent and titrating with bromine (*4*).

Monomers containing allyl and acrylate groups in the same molecule, such as allyl acrylate, cannot be analyzed by the authors' method because the low temperature and short period of time necessary to control substitution of the allyl group will prevent complete addition to the acrylate group. This problem has been studied by other investigators (*4*), however, and a procedure was evolved by them for estimating the amounts of allyl and acrylic unsaturation in a sample.

An excess of sulfuric acid must be added to the bromide-bromate sample solution to neutralize the pyridine and liberate the bromine. The amount of acid used should be about 75 to 100% of the theoretical amount in order to prevent the pyridine from reacting with an appreciable amount of free bromine.

The polymer absorbs bromine strongly, which makes it necessary to dissolve it in carbon tetrachloride before analyzing the

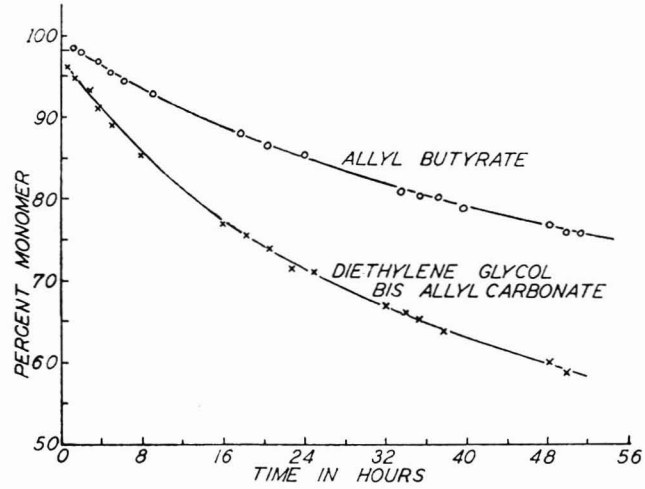

Figure 6. Polymerization of Allyl Butyrate and Diethylene Glycol–Bis(allylcarbonate)

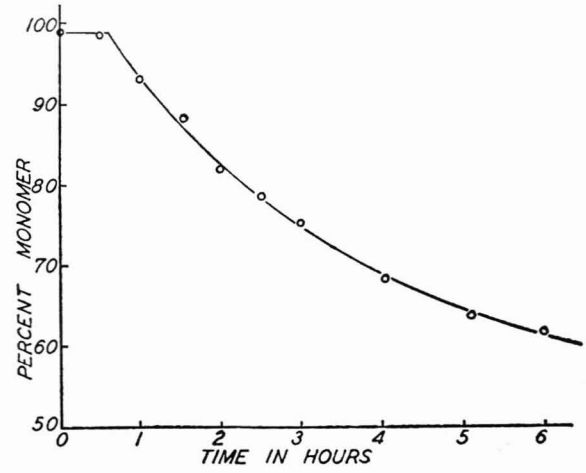

Figure 7. Polymerization of Isobutyl Acrylate

Table I. Analysis of Monomers

Monomer	Mole Br_2/Mole Monomer	% Monomer
Isobutyl acrylate	0.985	98.5
Ethyl methacrylate	0.989	98.9
Allyl butyrate	0.998	99.8
Diethylene glycol-bis(allylcarbonate)	1.948	97.4

Table II. Analysis of Synthetic Monomer-Polymer Mixtures

Compound		% Monomer
A.	Methyl methacrylate	98.7
B.	Isobutyl acrylate	98.5
C.	Polymer methyl methacrylate	3.1
D.	Polymer isobutyl acrylate	2.6

Mixtures		% Monomer	
		Analysis	Theory
75% A	25% C	74.5	74.8
50% A	50% C	49.7	50.9
75% B	25% D	74.8	74.5
50% B	50% D	49.6	50.5

solution for excess bromine. This absorption serves to enhance the addition reaction by concentrating bromine in the polymer.

The error of the analysis varies up to about 2%, depending upon the size of the sample, the aliquot portion taken, and the normality of the solutions employed.

EXPERIMENTAL RESULTS

Table I gives analytical data for commercial monomers which were vacuum distilled at 1-mm. pressure under a nitrogen atmosphere and analyzed for unsaturation immediately after distillation.

These analyses were all carried out in triplicate. The root mean square error for ten independent analyses of the diethylene glycol–bis(allylcarbonate) sample was ±0.287%.

Synthetic monomer-polymer mixtures were prepared to test the analytical procedure. The polymers were prepared according to Cohen *et al.* (*4*) from sirupy polymerized methyl methacrylate and isobutyl acrylate. The analyses of these polymers, the monomers, and their synthetic mixtures are given in Table II.

Figures 6 and 7 depict the rate of several bulk polymerizations carried out at 60° C. using 0.015 mole fraction of benzoyl peroxide as the polymerization agent. The unsaturation, expressed as per cent monomer (see Formula 1), is plotted against time.

ACKNOWLEDGMENT

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Streak Reagents for Chromatography

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A number of streak reagents, used in detecting zones of colorless substances on the chromatographic column, have been studied in regard to their usefulness in the detection of certain compounds. These reagents are all sensitive at concentrations of 0.01 molar. Several new streak reagents have been developed.

A NUMBER of methods have been developed for the detection of zones of colorless substances on a chromatographic column. Three of the most useful techniques are the detection of a zone by its fluorescence in ultraviolet light (*4, 8*), the detection by the formation of a dark zone on a fluorescent adsorbent (*1, 5*), and the detection by the use of streak reagents (*9*). Streak reagents are effective in concentration ranges where methods, such as refractive index changes, are often insensitive or difficult to apply, and are more broadly applicable than the other two methods mentioned. As a by-product of some work in this laboratory on the specificity of adsorbents the authors have acquired a considerable amount of information on the usefulness of these reagents on seven different adsorbents in detecting the following compounds: aromatic and aliphatic amines, alcohols, phenols, ketones, acids, aliphatic and aromatic nitro compounds, unsaturated hydrocarbons, aromatic compounds, and carbon disulfide. They present this information in the hope that it will be useful to workers who need to detect similar compounds.

Table I includes a summary of some streak reagents already reported. The results of the authors' work are summarized in Table II; data are given here for specific compounds on seven adsorbents and for two solvents, benzene and petroleum ether (60° to 70° C. boiling point). The tests in Table II were all satisfactory at 0.01 *M* concentration, which was selected because many tests are unsatisfactory at greater dilution. In all cases the initial volume of solution was πr^2 ml. (r = chromatographic tube radius). In some instances, where no suitable streak reagent was found, the application of other methods has been indicated. In general, it is desirable to have several streak reagents for a given type of compound, because it is not always possible to use a given reagent on all adsorbents—for example, acidic reagents are usually unsatisfactory on the carbonates. Spot test reactions and color reactions in general may be unsuitable for use on certain adsorbents or may require certain modifications before use, because of the effect of the adsorbent and the fact that very strong colors are required, owing to the thin film of liquid or of adsorbent surface available for color detection.

Some of the reagents used are new; others are adaptations of already existing tests as indicated by the literature references. Directions are included for preparation of these reagents. The reliability of these tests was checked by employing other detection methods to establish the presence of the substance sought in the zone detected.

Table I. Streak Reagents Found in Literature

Reagent	Zone Color	Compounds Detected	Lit. Ref.
Aqueous soln. of diazonium salt	Green or red	Benzidine or naphthylamine, naphthols	(9)
Lead peroxide in 30% acetic acid	Blue or green	Benzidine or naphthylamine	(9)
$KMnO_4$	Green	Many oxidizable compounds	(6, 9)
Schiff's reagent	Violet	Aldehydes	(6, 9)
Dimethylglyoxime	Red	Nickel sulfate	
$SbCl_3$ in chloroform	Blue	Vitamin A	(6, 9)
1% ceric sulfate in 85% sulfuric acid	Brown, blue, or red	Urea, urethanes, and related compounds	(7)
0.25% vanadium pentoxide in concd. H_2SO_4	Brown, blue, or red	Urea, urethanes, nitro compounds, and their derivatives	(7)
1% $K_2Cr_2O_7$ in concd. H_2SO_4	Brown and red	Same as previous test	(7)
6 *N* sodium hydroxide and 50% potassium hydroxide	Colored	Nitro compounds	(7)
a. 0.25% diphenylamine in concd. sulfuric acid *b.* 6 *N* sodium hydroxide[a]	Blue	Nitrates, *N*-nitroso, nitramine compounds	(7)
1% sodium nitrite in concd. sulfuric acid	Blue	Diphenylamine and related compounds	(7)
Griess test *a.* 6 *N* sodium hydroxide *b.* Aqueous soln. containing 30% acetic acid, 0.5% sulfanilic acid, 0.15% α-naphthylamine[a]	Weak pink	RDX, nitramines, and related compounds	(7)
Benzene-Franchimont test[a] *a.* Zn dust *b.* Benzene *c.* Griess solution	Pink	RDX[b], nitramines, related compounds	(7)
Schryver test[a] *a.* 1% phenylhydrazine hydrochloride in H_2SO_4 *b.* 5% aq. soln. of potassium ferricyanide	Red	RDX[b], and related compounds, formaldehyde	(7)
Saturated soln. of α-naphthylamine in concd. HCl *a.* Satd. aq. soln. of calcium hypochlorite *b.* 6 *N* NaOH[c]	Pink	*N*-Nitroso compounds	(7)
Concd. H_2SO_4	Colored	4-Nitroaniline	(7)
a. Concd. H_2SO_4[a] *b.* 6 *N* NaOH	Colored	Several types of compounds	(7)
Concd. HCl	Colored	Nitrosonitro compounds	(7)
Vongerichten's test[c] *a.* Satd. soln. of 2,4-dinitrochlorobenzene in alcohol *b.* 6 *N* NaOH	Colored	Some compounds	(7)
	Pink-orange	Pyridine	(7)

[a] Order listed indicates order in which reagents should be used. Streaks should be placed one upon the other.
[b] Hexahydro-1,3,5-trinitro-*s*-triazine (cyclonite).
[c] Order listed indicates order in which reagents should be used. Streaks should be placed adjacent to one another on the column but not touching.

PREPARATION AND USE OF REAGENTS

In the following section are given the preparation and method of application of each of the streak reagents listed in Table II. For convenience the tests are listed numerically.

1. **Alkaline Permanganate (6, 9).** This reagent is a 0.0075 *M* potassium permanganate solution in 0.25 *M* sodium hydroxide. The zone color is green and the blank column is purple.

2. **Diazonium Reagent.** This reagent is 1% aqueous solution of *p*-nitrobenzene diazonium fluoroborate. The zone is yellow to red on a colorless column.

3. **Zinc Dust and Alcoholic Sodium Hydroxide.** A pinch of zinc dust is added to a mixture of 1 ml. of 6 *N* sodium hydroxide and 1 ml. of ethyl alcohol and the mixture is heated just to boiling. This gives a yellow zone on a colorless column. It has been used only for nitrobenzene.

4. **Alcoholic Potassium Hydroxide and Nessler's Reagent.** Directions for the preparation of Nessler's reagent are given in all handbooks; the standard solution was used. The alcoholic potassium hydroxide is composed of equal parts of ethyl alcohol and 6 *N* potassium hydroxide. The column is first streaked with the potassium hydroxide and then overstreaked with Nessler's solution; carbon disulfide forms a yellow zone.

5. **BDH Universal Indicator.** This indicator can be used for some amines and acids. The color change is red for acids and green for bases.

6. **Bromine in Carbon Tetrachloride.** A 5% solution of bromine in carbon tetrachloride is the reagent used. It is useful for some amines. A colored section of the column indicates the zone.

Table II. Tabulated Streak Tests and Other Detection Methods Used to Locate Adsorbate Zones

No.	Adsorptive	Special Filtrol		Silicic Acid		Florisil		MgO		$CaCO_3$		$Ca(OH)_2$		$CaHPO_4 \cdot 2H_2O$	
		Bz[a]	P.E.[b]	Bz	P.E.	Bz	P.E.	Bz	P.E.	Bz	P.E.	Bz	P.E.	Bz	P.E.
1	1,3-Pentadiene	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Cyclohexene	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	*n*-Butylamine	1	1, 2	7	2	2, 11	1	2	2	5	5	11, 12	11, 12	2	2
4	Diethylamine	...	1, 2, 17	...	1	1	1	1, 2	1, 2	...	1	...	6, 12	..	2
5	Triethylamine	1	1	7	2	1, 6	1	1	1	1, 5	5	1	1, 12	..	1
6	Aniline	1, 2	1, 2, 17	1	1, 2	1, 2	1, 6	1, 2	1, 2	1	1, 18	1	1	2	2
7	Ethylaniline	...	1, 2, 17	...	1, 2	1, 6	1, 6	1, 2	1, 2	...	1	...	6	..	2
8	Diethylaniline	...	1, 2, 17	...	1, 2	1, 6	1, 6	1, 2	1, 2	...	1	...	1	..	1
9	Dimethylaniline	1, 2, 17	1, 2, 17	1	1, 2	1	1, 6	1, 2	1, 2	1	1	1	1	2	2
10	Diphenylamine	...	17	...	1, 2	1, 6	6	1, 2	1, 2	...	1	...	1	..	2, 14
11	Triphenylamine	17	17	...	1, 2	1, 6	6	6	6	...	6, 18	...	12	14	2, 14
12	Methanol	1	1	8	15	1	1	1	1	1	1	...	...	..	1
13	Ethyl alcohol	1	1	...	15	1	1	1	1	1	1	1	1	1	1
14	Phenol	1, 2	1, 2	1	1	1, 2	1, 2	1, 2	1, 2	1, 2	1	1	1	2	2
15	Acetone	1	1	1, 9, 16	1, 9, 16	1, 9, 16	9, 16	1	1	1	1	1	1	1	1
16	Anisole	1	1	...	...	1	1	1	18	1	18	1	1	1	18
17	Chlorobenzene	1	18	...	18	...	...	...	17	1	18	...	...	..	18
18	Acetic acid	1	1	8	5	1	1	1	...	...	...	...	...	..	...
19	Benzoic acid	18	1, 18	8	5	...	1	1	...	5	...	...	...	..	...
20	Nitromethane	1	1	1, 9	1	1	1	1	1	1	1	1	1	1	1
21	Nitrobenzene	3	3	3	3	3	3	3	3	3	3, 18	...	...	3	3
22	Benzene	...	18	...	...	...	...	...	17	...	18	...	...	..	18
23	Carbon disulfide	4	19	10, 13	13	13	4	4	4	4	4	4	4	13	19

[a] Benzene.
[b] Petroleum ether.
1. $KMnO_4$.
2. Diazonium.
3. Zn dust + alc. NaOH.
4. Methanoic KOH and Nessler's.
5. BDH Universal indicator.
6. Br_2 in CCl_4.
7. Nickel dimethyl glyoxime.
8. Iodide-starch-bromate.
9. Negative Nessler's test.
10. Azide-starch, iodide.
11. Schiff's and Nessler's.
12. Copper sulfate followed by bromine fumes.
13. Formalin and plumbite.
14. Special Filtrol suspension.
15. CS_2, 10% KOH, and Nessler's.
16. 2,4-Dinitrophenylhydrazine in HCl.
17. Visual.
18. Spectroscopic.
19. Olfactory.

7. **Nickel Dimethylglyoxime Reagent (2).** A 0.1 solution of nickel sulfate is saturated with dimethylglyoxime and the supernatant liquid is used as the streak reagent. It is useful for very basic substances. The zone is red on a colorless column.

8. **Iodide-Starch-Bromate Reagent.** The reagent is prepared by mixing equal volumes of 1% potassium bromate, 1% potassium iodide, and 5% starch solutions just before using. It is useful as test for acids. A dark blue zone is a positive indication.

9. **Negative Nessler's Test.** Nessler's reagent is streaked on the column and then overstreaked with 1 *M* ammonia. The inhibition of the orange color due to the reaction of the Nessler's reagent and the ammonia indicates a zone of acetone or nitromethane.

10. **Azide-Starch-Iodide Reagent (2).** This is prepared by mixing equal volumes of saturated solution of iodine in 1% potassium iodide and a solution of 10% sodium azide in 1% starch. A bleaching of the blue color indicates a zone of carbon disulfide.

11. **Schiff's and Nessler's Reagent.** The column is first streaked with Schiff's reagent and after 2 minutes is overstreaked with Nessler's reagent. A blue zone indicates some aliphatic amines.

12. **Cupric Sulfate and Bromine Fumes.** The column is streaked with the 0.1 *M* cupric sulfate solution, then exposed to fumes from a dilute solution of bromine in carbon tetrachloride passed over the column in a medicine dropper. The blank portions of the column along the cupric sulfate streak turn brown while the zone remains blue. This test is useful for di- and triarylamines.

13. **Formaldehyde and Plumbite Test (2).** The column is first streaked with a 40% solution of formaldehyde and followed by a streak of 0.1 *M* sodium plumbite; a brown zone indicates carbon disulfide.

14. **Special Filtrol.** A suspension of special Filtrol in petroleum ether or benzene is prepared. This suspension is streaked on the column so that there is a streak of the special Filtrol powder deposited. This powder will form a green or blue zone with many amines.

15. **Nessler's–Potassium Hydroxide–Carbon Disulfide Reagent.** The column is streaked with carbon disulfide and overstreaked with 10% potassium hydroxide, which in turn is overstreaked with Nessler's reagent. The formation of a yellow zone indicates an alcohol.

16. **2,4-Dinitrophenylhydrazine in Hydrochloric Acid (3).** A saturated solution of 2,4-dinitrophenylhydrazine in 2 *M* hydrochloric acid gives a strong yellow zone on a pale yellow background with ketones and aldehydes. On overstreaking with 6 *M* sodium hydroxide a transient dark color develops.

The following reagents have not been found in the literature and are apparently new in their application as streak tests: 2, 3, 4, 8, 9, 11, 12, 14, 15, and 16.

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Characterization of Some Chromatographic Adsorbents

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Data are presented here for the characterization of a number of chromatographic adsorbents. The relation between T_{50} and V_c is derived and confirmed by experimental data.

THE work reported here is part of a program of study of the specificity of chromatographic adsorbents. It was necessary to know the characteristics of the common adsorbents in order to select those suitable for use. As a by-product of this investigation some interesting relations have been shown between the adsorbent and its properties.

The methods used are described elsewhere (*2*); they consisted essentially of determining for each adsorbent the characteristic values of V_c, and T_{50}, and measuring adsorption affinity. Other terms such as k and K could be calculated from these data.

DEFINITION OF TERMS

V_c = rate of flow of the developing solvent through the chromatographic column measured in mm. per minute at constant flow. The values given are for columns 75 ± 2 mm. long packed under vacuum alone. The driving pressure was about 700 mm. of mercury. The vacuum was supplied by a water pump. The packing is aided only by tapping the glass tube with the stamper until no more settling is observed

T_{50} = time in seconds required for the solvent to penetrate 50 mm. into an initially dry column 75 ± 2 mm. long under a driving force of a pressure of about 700 mm. of mercury (the full vacuum supplied by a water pump). In the present work benzene was used as the solvent

k = permeability of column in darcys

K = a constant formed by the grouping of constants ($kA/760\alpha$). This constant is useful in estimating the flow rates through other types of column ($V_c = KP/\eta L$)

A = cross-sectional area of the column, sq. cm.

P = driving pressure, mm. mercury

α = column interstitial volume, ml. per mm.

η = viscosity of solvent, centipoises

L = column length, cm.

S = a packing measure defined as the ratio of the length of packed column required to hold a unit volume of solvent to the length of unpacked tube required to contain the same volume of solvent

x = distance of a point on an adsorptive zone from the top of the column (usually the leading or trailing edge of the zone)

D = distance the front edge of the developing solvent has moved in the packed column, measured from the top of the column

R = ratio of the movement of an adsorbed zone to the movement of the solvent defined mathematically as dx/dD. The rate for the leading edge is R_l and for the trailing edge is R_t. For this determination the initial volume of solution was πr^2 ml. of 0.01 *M* solution (r is radius of tube used)

The experimental data are shown in Table I; blanks indicate that the data were not obtained because of difficulty in measurement. On prewashed columns T_{50} could not be obtained and in general calculations were not made for k and K. On some adsorbents the *o*-nitroaniline zone could not be detected because of the dark color of the adsorbent, etc. Attempts to use fluorescent substances for the R determination on these adsorbents were not successful because the fluorescent zone could not be observed.

Table I. Characteristics of Some Chromatographic Adsorbents

Adsorbent	V_c, Mm./Min.	T_{50}, Sec.	$K \times 10^2$ (kA/760 α)	k, Darcys	Ml./Mm.	S	*o*-Nitroaniline[a] R_f	*o*-Nitroaniline[a] R_t
Silicic Acid and Silica Gel								
Reagent silicic acid, Merck								
lot 1[b]								
Untreated	10.0	115	6.2	3.1	0.039	1.62	0.30[c]	0.20[c]
Prewashed	17.0	...	...	...	...	...	0.18	0.10
Reagent silicic acid, Merck lot 2[b]								
Untreated	3.4	258	2.13	1.12	0.043	1.48	0.59	0.48
Prewashed	5.2	...	...	...	...	...	0.24	0.13
Silicic acid, Baker								
Untreated	7.1	196	3.1	1.5	0.042	1.51	0.45	0.39
Prewashed	...	...	...	...	...	...	0.11	0.02
Silica gel, Davidson								
325-mesh	21.0	44	13.8	6.34	0.041	1.64	0.08	0.05
Silicates								
Silene E.F.	7.1	182	3.8	2.3	0.051	1.25	0.56	0.37
Florisil								
30–60-mesh	51[d]	24[d]	246	115	0.040	1.63	0.40	0.14
60–100-mesh	32[c]	...	153	79	0.044	1.46	0.39	0.21
100–200-mesh	8.9	53	43	23.2	0.044	1.41	0.29	0.18
200–300-mesh prewashed	9.2	123	6.02	3.86	0.043	1.48	0.07	0.07
Florex, 200–300-mesh	14.6	87.1	9.53	5.12	0.046	1.41	...	...
Calcined florex, 200–300-mesh	8.8	146	5.64	3.07	0.046	1.40	...	...
Super Filtrol	10.9	96	6.8	2.9	0.036	1.77	0.01	0.01
Special Filtrol	10.8	100	7.2	3.13	0.039	1.76	0.15	0.14
Lloyd's reagent	15.4	86	10.1	3.3	0.031	2.22	...	...
Fuller's earth	11.6	100	7.4	4.0	0.048	1.41	(0.39)	(...)
260 clay	12.9	85	8.4	3.48	0.035	1.81	...	...
Alumina								
Anhydrous, E. & A.	17.1	84	11.1	5.4	0.041	1.57	0.87	0.73
Ignited, Baker	138	8.3	88.6	42.3	0.040	1.58	0.69	0.65
Old sample	...	...	...	...	0.041	1.56	0.55	0.51
New Sample								

[a] Benzene used as developing solvent for R_f determination and also for V_c and T_{50}.
[b] Two separate shipments of this adsorbent, illustrating variation in properties of different batches.
[c] Old and questionable data.
[d] 15-mm. benzene head without aid of suction pump was only driving force (≡ 0.97 mm. Hg).

Adsorbent	V_c, Mm./Min.	T_{50}, Sec.	$K \times 10^2$ (kA/760 α)	k, Darcys	Ml./Mm.	S	*o*-Nitroaniline[a] R_f	*o*-Nitroaniline[a] R_t
Alumina (*Contd.*)								
Aluminum Ore Co., 80-mesh								
F20	...	...	...	...	0.037	1.75	0.49	0.45
2200	76	29.6	49.0	24.1	0.042	1.51	0.17	0.18
H-40	171	6	111	44.5	0.034	1.90	(0.3)	(0.3)
Florite	9.1	152	5.9	2.5	0.036	1.79	...	...
Lime								
Purified, Baker	23	47	15.1	7.5	0.042	1.53	...	...
Reagent, Baker	12	94.8	7.8	3.9	0.042	1.54	(1.0)	(1.0)
Miss. Lime Co.	23	50	14.9	7.1	0.041	1.58	...	...
Tiger brand, Kelly Island Lime Co.	2.9	293	2.2	1.4	0.043	1.49	...	...
Captain brand, Pure Lime Co.	14	82	9.3	4.7	0.043	1.49	...	...
Batesville, White Lime Co.	7.6	160	4.8	2.4	0.042	1.54	...	...
Calcium Carbonate								
Mallinekrodt	164	5.9	108	44.1	0.036	1.86	1.0	1.0
Heavy powder, Merck	28	40.5	17.8	7.8	0.038	1.78	1.0	1.0
Magnesium Oxide								
Heavy powder, Baker	7.5	136	4.5	2.6	0.048	1.30	0.34	...
Merck	3.2	306	2.1	1.23	0.049	1.32	0.19	0.14
Micron brand	1.58	505	1.03	0.50	0.041	1.55	0.35	0.19
Charcoal								
Norit A	4.3	275	2.6	1.3	0.041	1.54	Very small	
Mallinekrodt	1.92	399	1.24	0.64	0.045	1.42	...	...
Miscellaneous								
Zinc oxide, Baker	8.9	29.7	5.7	3.4	0.050	1.29	...	...
Zinc carbonate, Baker	10.7	121	6.9	4.45	0.55	1.32	1.0	1.0
Calcium oxide, Baker	27.8	31.8	17.7	9.55	0.045	1.40	0.93	0.93
$CaHPO_4$, Baker	23.4	42.3	15.2	7.27	0.040	1.58	1.0	1.0
$Ca_3(PO_4)_2$, Baker	19.9	51.0	13.2	6.5	0.042	1.53	0.90	0.82

Some adsorbents did not adsorb *o*-nitroaniline appreciably from benzene and consequently can only be considered as weaker adsorbents than the others in regard to the standard substance used here.

For ordinary work it is generally desirable that the values of V_c, k, K, and R_f be in the following ranges:

V_c = 10 to 50 mm. per minute (using benzene as a solvent)
k = 3 to 17 darcys
R_f = 0.10 to 0.30 for substance to be chromatographed
K = 0.06 to 0.32

Variations in S are of minor importance.

A large amount of data is made available here for a comparison between T_{50} and V_c. The relationship between these two quantities was derived, assuming constant pressure throughout the determination, which is approximately true. The result of this calculation was the equation: $V_c = 1000/T_{50}$. A comparison between the values found experimentally and the line given by plotting this equation is shown in Figure 1. The agreement is enough to justify the assumption that the relation is true, and therefore V_c values may be calculated from T_{50} determinations. The derivation of the relation is shown below:

The term V_c has been shown to be inversely proportional to the column length at constant solvent viscosity (*1*).

$$V_c = b/L \text{ or } 1/V_c = L/b \qquad (1)$$

Here b is a constant and L is the length of the column in millimeters

The term T_{50} may be represented as follows:

$$T_{50} = \int_0^{50} dL/V_c = \int_0^{50} LdL/b =$$

$$[L^2/2b]_0^{50} = \left(\frac{L}{2}\right)\left(\frac{L}{b}\right) = 50/2V_{c_{50}} \qquad (2)$$

In this step constant pressure was assumed; although this is not strictly true, the results indicate that it is a reasonable approximation. The $V_{c_{50}}$ differs from $V_{c_{75}}$ which is the value reported experimentally.

Inasmuch as V_c is determined for a 75-mm. column, it is necessary to convert $V_{c_{50}}$ into terms of $V_{c_{75}}$. Because V_c is inversely proportional to the column length, it follows:

$$V_{c_{50}} = (75/50)\ V_{c_{75}} = (3/2)\ V_{c_{75}} \qquad (3)$$

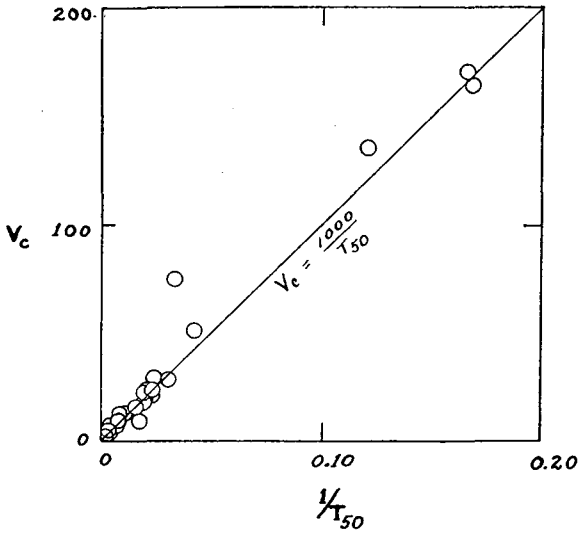

Figure 1. Theoretical and Experimental Values of Relation between V_c and $1/T_{50}$

V_c is given in minutes and T_{50} is given in seconds, so the final value for $V_{c_{50}}$ is:

$$V_{c_{50}}(\text{sec.}) = \left(\frac{3}{2 \times 60}\right) V_{c_{75}} \qquad (4)$$

and finally:

$$T_{50} = \frac{50}{2\left(\frac{3}{2 \times 60}\right) V_{c_{75}}} = 1000/V_c$$

where the last V_c is $V_{c_{75}}$.

It is interesting to compare some of the adsorbents in regard to their adsorption of the standard substance, *o*-nitroaniline, as shown in Table I. Many factors other than the surface structure of the adsorbent affect the adsorption affinity—viz., surface area, particle size, purity, adsorbed moisture, etc. It is evident that the order of decreasing adsorption affinity is roughly: acid silicates, silica gel, silicic acid, alkaline oxides, alkaline hydroxides. Magnesium-containing adsorbents apparently are stronger than the corresponding calcium compounds with reference to *o*-nitroaniline.

Data for lot numbers of the batches of adsorbents used here are not given, as it would be practically impossible to obtain these particular lots of adsorbent for future use. In general, it has been the authors' experience that the various brands of adsorbents show only minor variations in properties, whereas the only variations of major importance in the choice of an adsorbent are in order of magnitude; thus an adsorbent may show a flow rate which is too fast, too slow, or satisfactory for a given operation. In the authors' experience, Merck reagent silicic acid over a period of about 6 years has always shown nearly the same properties, regardless of the lot used; two lots are included in the present survey as an indication of the extent of variation which may occur from lot to lot. The cases used represent approximately the maximum variation noted for this particular adsorbent. Numerous other cases of this same kind might be cited. The data presented here have been found very useful in the selection of adsorbents. It is hoped that the publication of this kind of data will stimulate the characterization of commercial adsorbents and thereby aid in making available better adsorbents.

LITERATURE CITED

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Critical Examination of Platinum Sulfide Precipitation

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A detailed investigation has been made of the conditions affecting the sulfide precipitation of platinum, in particular the effect of various proportions of sodium chloride and of hydrochloric acid. The efficiency of different concentrations of ammonium chloride and of hydrochloric acid used to wash and leach the precipitate has been evaluated. A procedure for the accurate microdetermination of platinum in the presence of sodium chloride has been developed.

THE precipitation of a platinic salt with hydrogen sulfide was recorded by Berzelius in 1826 (*4*). Many early workers were of the opinion that the precipitated material was complex in nature. Antony and Lucchesi (*1*) stated that at temperatures below 90° C. sulfoplatinates were formed. However, von Meyer (*11*) considered that the precipitate was a loose compound of platinum disulfide and hydrogen sulfide. These two concepts have been discussed by recent investigators (*2, 5, 9, 13*) in the light of modern theories of structure, but no completely satisfactory picture has been evolved.

DETERMINATION OF PLATINUM BY STANDARD PROCEDURES

Three generally accepted recipes for the determination of platinum by means of the sulfide precipitation have been reported (*7, 8, 16*). These procedures differ significantly in detail. It has long been accepted in the authors' laboratory that all these procedures produced high results and that there was an apparent discrepancy in the results obtained from different procedures. The hydrogen sulfide precipitation of platinum is preceded by a separation from interfering elements. Sodium chloride is therefore a probable constituent of the precipitation medium. This condition was anticipated in the preparation of the standard platinum solution. A weighed quantity of reagent platinum was dissolved in aqua regia, sodium chloride was added, and the solution was evaporated repeatedly in the presence of concentrated hydrochloric acid. Each aliquot used contained 0.10 gram of sodium chloride, giving, before precipitation, a solution 0.034 molar in sodium chloride. The platinum in

Table I. Platinum Recovered by Three Standard Procedures

Method	Platinum Added		Platinum Recovered	Error
	Mg.		*Mg.*	%
Treadwell and Hall	10.12		10.33	+1.2
			10.33	1.2
			10.31	1.0
			10.34	1.3
			10.33	1.2
			10.28	0.7
			10.29	0.8
		Av.	10.31	1.1
Gilchrist and Wichers	10.12		10.29	+1.6
			10.28	1.5
			10.27	1.4
			10.23	1.0
			10.24	1.1
			10.23	1.0
			10.22	0.9
		Av.	10.25	1.2
Hillebrand and Lundell	10.12		10.36	+2.3
			10.26	1.3
			10.38	2.5
			10.30	1.7
			10.38	2.5
			10.33	2.0
			10.25	1.2
		Av.	10.32	1.8

this standard solution was determined by each of the above methods.

Table I contains data obtained for this report. These results support the long experience of one of the authors that the standard procedures produce results lacking in the desired accuracy. The present investigation, undertaken to determine the cause of these high results, formed part of a detailed survey of methods for the determination of platinum.

REAGENTS

Standard platinum solutions were prepared by dissolving a weighed quantity of Baker reagent grade platinum metal in aqua regia and evaporating to dryness repeatedly with and without the addition of sodium chloride. The standard solution was prepared by dissolving the above residue to a known volume. Spectroscopic examination of evaporated portions of the standard solutions indicated only traces of impurity. The calculated platinum content of the solution was checked by a formate precipitation (*3*).

Hydrogen sulfide gas, supplied by the Matheson Company, East Rutherford, N. J., was passed through water and air traps to remove mechanical impurities.

Reagent grade chemicals were used.

EFFECT OF SODIUM CHLORIDE AT SEVERAL HYDROCHLORIC ACID CONCENTRATIONS

Preliminary experiments showed that the accuracy obtained depended, partly at least, on the concentrations of sodium chloride and hydrochloric acid.

Measured amounts of hexachloroplatinic acid prepared from platinum together with a solution of sodium chloride were evaporated to dryness on the steam bath. The evaporated material was diluted to 50 ml. in all cases and the acidity adjusted. The solutions were heated to the boiling point and "gassed" with hydrogen sulfide for 30 minutes, cooled, filtered through a Whatman No. 42 paper, and washed with 1% ammonium chloride solution, and the precipitate was ignited at 800° C. for 1 hour. After the residue was cooled in air, it was leached with 1% ammonium chloride, followed by a second ignition at 800° C. for 1 hour. The resulting sponge was cooled and weighed under conditions of constant humidity (*15*). A blank was subtracted. All filtrates gave negative stannous chloride tests indicating complete precipitation.

The results are recorded in Figure 1. Weights of residues obtained are expressed as percentage ratios of the amount of platinum taken. Sufficient determinations, at least five, were made to give a precision within at least ±0.2% as measured by the average deviation. The results indicate that the accuracy obtained in the sulfide precipitation depends upon the concentration of both sodium chloride and hydrochloric acid. Furthermore, the curves indicate that the deviations are not fully explained as occlusion or adsorption phenomena. Curves *B* and *C* show an optimum concentration of sodium chloride and hydrochloric acid.

By analogy with results obtained in other precipitations (*14*) it was felt that the order of addition of reagents, together with any treatment previous to precipitation, should have a pronounced effect on the purity of the precipitate obtained.

EFFECT OF ORDER OF ADDITION OF REAGENTS

Experiment 1. It is customary when precipitating platinum by means of hydrogen sulfide to pass the gas through the acidified and heated platinum solution. The effect of adding the hexachloroplatinic acid to a hydrochloric acid solution saturated with respect to hydrogen sulfide was investigated. To 40 ml. of the hydrochloric acid solution saturated with hydrogen sulfide were added 10 ml. of hexachloroplatinic acid solution. The combined solution was 1.0 molar in hydrochloric acid. After heating on the steam bath for 1 hour 15 ml. more of the acidified hydrogen sulfide solution were added and the heating was continued for 1 hour.

Experiment 2. To 40 ml. of the acidified hydrogen sulfide solution were added 10 ml. of hexachloroplatinic acid solution previously evaporated in the presence of sodium chloride. Precipitation was effected as outlined above.

Experiment 3. To 40 ml. of the acidified hydrogen sulfide solution were added various amounts of solid sodium chloride and 10 ml. of hexachloroplatinic acid solution with no evaporation. Precipitation was effected as outlined above.

Experiment 4. A solution of total volume 50 ml., 1.0 molar in hydrochloric acid containing hexachloroplatinic acid and 1.0 gram of sodium chloride not previously evaporated in the presence of the hexachloroplatinic acid, was heated to the boiling point and "gassed" in the conventional manner.

Experiment 5. Two standard solutions were prepared by

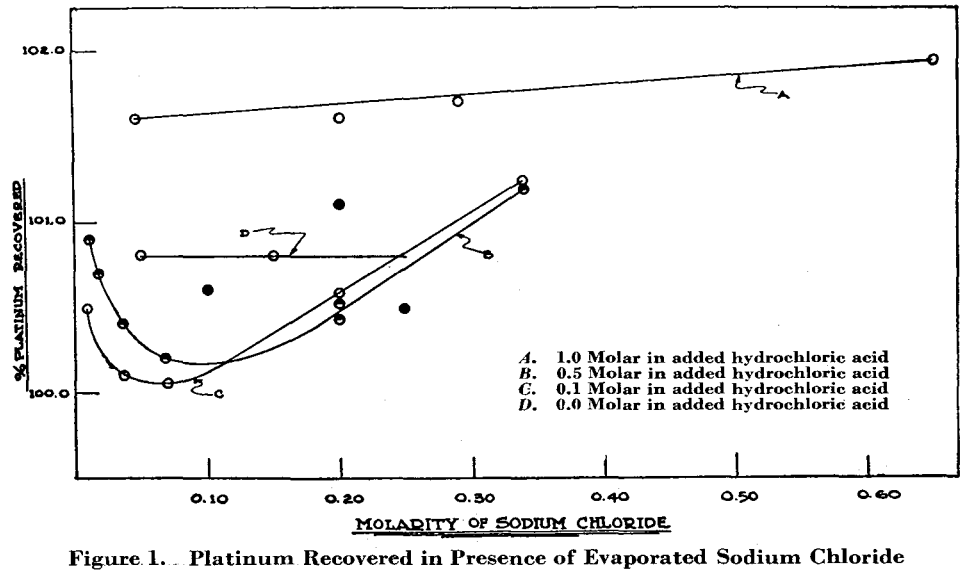

Figure 1. Platinum Recovered in Presence of Evaporated Sodium Chloride

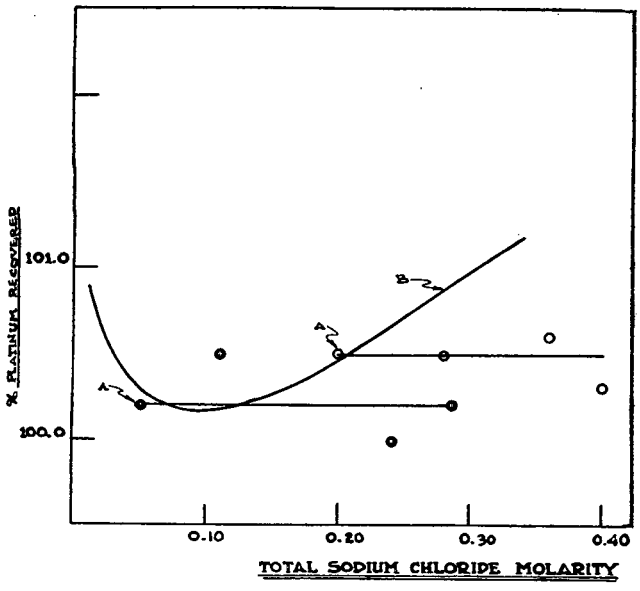

Figure 2. Platinum Recovered in Experiment 5

A. **Concentration of sodium chloride evaporated in presence of hexachloroplatinic acid**
B. **Curve *B* from Figure 1**

evaporating hexachloroplatinic acid in the presence of sodium chloride and diluting to a known volume. To 10-ml. aliquots of these solutions containing approximately 10 mg. of platinum were added varying amounts of solid sodium chloride and 40 ml. of a hydrochloric acid solution saturated with hydrogen sulfide. The combined solution was 0.5 molar in hydrochloric acid. Precipitation was carried out on the steam bath. Sufficient determinations, at least five, were made at each sodium chloride concentration to give a precision as measured by the average deviation of ±0.2%. The platinum recovered was expressed as a percentage of the platinum taken and plotted against the total sodium chloride content of the solution. Data previously obtained for hexachloroplatinic acid evaporated in the presence of sodium chloride and precipitated from a solution 0.5 molar in hydrochloric acid (Figure 1) are superimposed on the results of this experiment.

Table II. Effect of Order of Addition of Reagents

Expt. No.	Molarity HCl	Molarity NaCl	Platinum Taken *Mg.*	Platinum Recovered *Mg.*
1	1.0	...	9.98	9.95
				9.99
				9.98
				9.97
				10.03
2	1.0	0.20	9.99	10.16
				10.14
3	1.0	0.034	9.98	9.98
				9.98
				9.96
				9.97
	1.0	0.17	9.98	9.98
				9.98
				9.98
				9.97
				10.02
	1.0	0.34	9.98	9.96
				10.00
				10.03
	1.0	0.51	9.98	10.02
				10.02
				10.05
				10.11
				10.09
				9.95
				10.01
				10.08
4	1.0	0.34	9.98	10.15
				10.14
				10.14
				10.14
				10.11

Data from these experiments are given in Table II and Figure 2. These results indicate that the hydrogen sulfide precipitation from hexachloroplatinic acid does not require prolonged gassing. This does not support the generally accepted opinion that prolonged contact with hydrogen sulfide is required (*10*, *12*). Furthermore, it is evident that evaporating the hexachloroplatinic acid in the presence of sodium chloride leads to high results. This effect is not obtained by using a procedure involving no evaporation or boiling.

The material obtained by evaporating hexachloroplatinic acid in the presence of sodium chloride is probably not a simple substance. Genke (*6*) has shown that at least two chemical species are present. In the opinion of the authors some type of complex formed between sodium chloride and platinum(IV) chloride may best explain the results obtained in this research. If this is true, "displacement" of the "complexed" sodium chloride should lead to greater accuracy.

DISPLACEMENT OF SODIUM CHLORIDE

By Hydrochloric Acid. Samples of hexachloroplatinic acid were evaporated to dryness with sodium chloride and the residue was taken up with 50 ml. of concentrated hydrochloric acid. A considerable portion of sodium chloride remained insoluble, but the color of the solution indicated that the greater part of the platinum compound had dissolved. The mixture was placed on a hot plate and gassed while the temperature was slowly raised from room temperature to the boiling point. Reaction occurred readily under these conditions. Hydrochloric acid was given off during the heating process and as the temperature approached the boiling point the excess sodium chloride dissolved. With a sodium chloride molarity of 0.08 there was a recovery of 100.9% and with a molarity of 0.60 the recovery was 102%.

Evidently strong hydrochloric acid does not displace the sodium chloride to the desired degree, although a comparison of results in Figure 1 and Table II suggests that some "displacement" has taken place at the lower sodium chloride concentration.

By Ammonium Chloride. METHOD 1. To a solution of hexachloroplatinic acid containing approximately 10 mg. of platinum was added solid sodium chloride and the mixture was evaporated to dryness on the steam bath. The residue was taken up with 10 ml. of distilled water, solid ammonium chloride was added, and the mixture was again evaporated to dryness. Twenty milliliters of hydrochloric acid solution and 30 ml. of water saturated with hydrogen sulfide were added and the combined solution was precipitated on the steam bath.

METHOD 2. To a hexachloroplatinic acid solution were added solid sodium chloride and ammonium chloride and the mixture was evaporated to dryness on the steam bath. Hydrochloric acid and hydrogen sulfide were added as in Experiment 1 and precipitation was effected on the steam bath.

METHOD 3. A hexachloroplatinic acid solution was evaporated in the presence of sodium chloride and ammonium chloride as in Experiment 2. The evaporated residue was taken up with dilute hydrochloric acid and the mixture was heated to boiling. Hydrogen sulfide gas was passed in for 30 minutes.

The results of these experiments, all of which were obtained consecutively, are given in Table III. These results show that ammonium chloride is an efficient reagent for the removal of interference, provided it is added to the solution before evaporation.

Washing and Leaching Reagents. The authors' work suggests that while the cause of high results is not entirely adsorption, nevertheless this phenomenon does play a part, and leaching is always necessary. Certain standard procedures use no leaching process and there are no data dealing with its quantitative effect. It was considered desirable to ascertain in a systematic fashion the effect of several washing and leaching reagents.

A standard platinum solution was prepared by dissolving platinum metal in aqua regia, adding sodium chloride, and evaporating repeatedly to dryness in the presence of hydro-

Table III. Effect of Ammonium Chloride

Expt. No.	Molarity NaCl	Molarity NH_4Cl	HCl	Platinum Taken *Mg.*	Platinum Recovered *Mg.*
1	0.34	0.74	...	10.00	10.13
		0.74	...	10.00	10.12
		0.74	0.5	10.00	10.03
		0.74	0.5	10.00	10.05
		0.74	0.5	10.00	10.02
		0.81	0.5	10.03	10.10
		0.81	0.5	10.03	10.20
		0.81	0.5	10.03	10.17
		0.74	1.0	10.00	10.09
		0.74	1.0	10.00	10.16
		0.74	1.0	10.00	10.13
		0.74	1.0	10.00	10.09
		0.74	2.0	10.00	10.04
		0.74	2.0	10.00	10.08
2	0.17	0.37	0.5	10.00	10.00
	0.17	0.37	0.5	10.00	10.01
	0.34	0.37	0.5	10.00	10.02
	0.34	0.37	0.5	10.00	9.99
	0.34	0.74	0.5	10.03	10.03
	0.34	0.74	0.5	10.03	10.03
	0.68	0.74	0.5	10.03	10.03
	0.68	0.74	0.5	10.03	10.04
	0.34	0.74	1.0	10.03	10.08
	0.34	0.74	1.0	10.03	10.11
	0.34	0.74	1.0	10.03	10.05
3	0.34	0.74	0.5	10.03	10.04
	0.34	0.74	0.5	10.03	10.02

chloric acid. To an aliquot of the above solution containing 10.20 mg. of platinum and 0.1 gram of sodium chloride were added sufficient hydrochloric acid and water to make a solution of total volume 50 ml., 0.1 molar in hydrochloric acid. Hydrogen sulfide was passed in for 30 minutes after heating to the boiling point. The precipitate was filtered through a No. 42 Whatman filter paper. The precipitate and paper were ignited at 800° C. for 1 hour, free access of air being assured at all times. The roasted sponge was leached by placing the crucible in a small beaker and dislodging the precipitate with a stream of reagent from a wash bottle. The contents of the beaker were then brought to the boiling point and the crucible was removed and washed with distilled water. The leached sponge was filtered and ignited at 800° C. for 1 hour. Cooling and weighing were carried out as previously described.

An estimate of the sulfur content of the ignited sponge was made by dissolving approximately 50 mg. of the sponge in aqua regia under a trap containing aqua regia. After the combined solutions were evaporated to dryness repeatedly with hydrochloric acid, the residue was taken up with dilute hydrochloric acid and the sulfate present was precipitated as barium sulfate. The results are given in Table IV. In all cases sufficient experiments were performed to give a precision of ±0.2% as measured by the average deviation.

Inspection of the results indicates that leaching with either hydrochloric acid or ammonium chloride gives more accurate results. The washing agent used makes no appreciable difference. There appears to be little relation between the sulfur content of the sponge and the accuracy obtained. In all work reported a wash and leach of 1% ammonium chloride was used because a slightly greater precision was obtained. In each case qualitative examination of the filtrates from leaching gave positive tests with zinc uranyl acetate reagent, indicating the presence of sodium ion. Blanks using filter paper gave negative tests under similar conditions. The sodium chloride and hydrochloride acid concentrations chosen were the optimum concentrations as shown in Figure 1.

NATURE OF THE PRECIPITATE

An attempt was made to elucidate the mechanism of the precipitation, especially with reference to the results obtained. The sulfide precipitates obtained from hexachloroplatinic acid solutions were analyzed.

The precipitates were formed by adding 40 ml. of a saturated aqueous solution of hydrogen sulfide, 0.1 molar in hydrochloric acid, to 10 ml. of a hexachloroplatinic acid solution containing 10 mg. of platinum. The precipitates were washed with water, ethyl alcohol, pyridine, and ether in the order named and dried at 100° C. The weight of residue after ignition at 800° C. for 2 hours was taken as the platinum content. In order to arrive at the sulfur content, a weighed sample prepared as outlined above was dissolved in aqua regia under a trap containing aqua regia. After repeated evaporations with hydrochloric acid the sulfate present was precipitated with barium chloride.

The results are given in Table V under Experiments 1 and 2. In an inert atmosphere of nitrogen these precipitates evolved hydrogen sulfide when heated between 150° and 250° C. An analysis was made of the precipitate after prolonged heating to 250° C., under nitrogen (Table V, Experiment 4). The latter analysis corresponds to a formula PtS_2, whereas the previous analysis corresponds to the formula $PtS_2.H_2S$. Analyses of precipitates obtained from samples of hexachloroplatinic acid evaporated in the presence of sodium chloride were, in general, unsatisfactory, lacking reproducibility. The result of one such analysis is given under Experiment 3. Inspection of the results indicates that the composition of the material precipitated from hexachloroplatinic acid in the absence of "complexed" sodium chloride may be represented as PtS_2 with associated hydrogen sulfide.

Examination of the material precipitated in the absence of sodium chloride by means of x-ray diffraction powder techniques showed that it lacked any crystalline characteristics. Precipitates obtained from hexachloroplatinic acid evaporated to dryness with sodium chloride showed evidence of crystalline structure. Measurements of line spacings indicated that the material producing the lines was not sodium chloride nor platinum disulfide.

Table IV. Effect of Washing and Leaching

Washing Reagent	Leaching Reagent	Weight of Ignited Residue *Mg.*	Weight of Sulfur as S *Mg.*
0.1 *M* HCl	.....	10.46	0.06
0.1 *M* HCl	Water	10.27	0.01
0.1 *M* HCl	0.1 *M* HCl	10.24	0.02
0.1 *M* HCl	0.5 *M* HCl	10.24	0.02
0.1 *M* HCl	1.0 *M* HCl	10.21	0.02
0.1 *M* HCl	1% NH_4Cl	10.25	0.01
0.1 *M* HCl	10% NH_4Cl	10.25	0.02
Water	1% NH_4Cl	10.26	0.04
0.1 *M* HCl	1% NH_4Cl	10.25	0.01
0.5 *M* HCl	1% NH_4Cl	10.23	0.01
1.0 *M* HCl	1% NH_4Cl	10.24	0.01
1% NH_4Cl	1% NH_4Cl	10.23	0.01
10% NH_4Cl	1% NH_4Cl	10.25	0.02

Table V. Analysis of Sulfide Precipitates

Expt. No.	HCl Concn., Molarity	NaCl Concn., Molarity	Pt, %	S, %	Na, %
1	1.0	...	63.8	35.3	...
2	1.0	...	64.1		...
3	1.0	0.17	65.6	30.4	1.6
4	1.0	...	71.9	28.0	...

CONCLUSIONS

Precipitation of platinum sulfide from a solution previously evaporated in the presence of sodium chloride produces high results. The presence of sulfur in the precipitate does not account for this error.

The accuracy obtained using samples of hexachloroplatinic acid previously evaporated with sodium chloride depends on the concentration of sodium chloride and hydrochloric acid.

Accurate results may be obtained by evaporating simultaneously in the presence of sodium chloride and ammonium chloride.

Accurate results are obtained in the presence of sodium chloride if there has been no evaporation or boiling with hexachloroplatinic acid.

The hydrogen sulfide precipitation does not require prolonged gassing.

Leaching the ignited sulfide precipitate increases the accuracy of results.

The material precipitated from hexachloroplatinic acid by hydrogen sulfide contains platinum sulfide together with hydrogen sulfide not soluble in water but volatile at 150° to 250° C.

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Investigation of Paper Strip Chromatography

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Twenty-two filter papers were examined for use in paper strip chromatography using mixtures of amino acids and five different mixed-solvent developers. Fifty-three other papers were examined with two mixed-solvent developers. A brief report is made of the results.

PAPER strip chromatography (*4*, *5*, *9*) is widely used as an analytical tool. A variety of filter papers are available, but little information on their usefulness in this technique has been published, though some comparisons have been made (*3*, *5*). This communication summarizes the results of a comparison of twenty-two filter papers against five solvents in the separation of test mixtures of amino acids. A brief report of the more noteworthy phenomena observed is given here without the mass of data which a full report would require.

EXPERIMENTAL

Filter Papers. The filter papers tested were from Reeve Angel (R.A.), Nos. 201, 202, 204, 211, 226, 230; Schleicher and Schuell (S.S.), Nos. 507, 576, 589 Blue Ribbon, 589 Black Ribbon, 589 White Ribbon, 589 Red Ribbon (tested in only one solvent), 595, 597, 598, 602, 604; and Whatman (W.), Nos. 1, 2, 3, 4, 5. These were purchased in sheets, and were assumed to represent the average quality of these papers.

Solvent Mixtures. Collidine was obtained by distillation of a crude mixture of trimethylpyridine isomers, taking the fraction 46° to 63° C. at 10 mm. This was saturated with distilled water.

Phenol 80%, water 20%, by weight.

1-Butanol saturated with 3% aqueous ammonia.

2-Butanol 75 parts, 90% formic acid 15 parts, water 10 parts by volume.

2-Butanol 90 parts, 3% aqueous ammonia 40 parts by volume.

Amino Acid Mixtures. Particular amino acid mixtures were prepared for use with each solvent mixture such that different types of acids were present—e.g., acidic, basic, aliphatic, and aromatic—and some pairs of amino acids with similar R_F values were present to test separability. The mixtures used were:

With collidine: aspartic acid, proline, histidine, valine, and phenylalanine

With phenol: aspartic acid, glycine, hydroxyproline, arginine monohydrochloride, valine, and phenylalanine

With 1-butanol–ammonia: same mixture as with collidine

With 2-butanol–formic acid: leucine, hydroxyproline, arginine monohydrochloride, phenylalanine, and glutamic acid

With 2-butanol–ammonia: leucine, hydroxyproline, aspartic acid, histidine, and phenylalanine

Procedure. The method of descending paper strip chromatography (*4*) was followed, a standardized procedure being used. Filter paper was cut in the machine direction in strips 9 cm. wide. Spots of amino acid mixture (2 μl. per spot) were applied in the presence of ammonia vapor along a line 10 cm. from one end of the strip, never closer than 2.5 cm. from the edge of the strip, and allowed to dry. The strips were then aligned in a trough (*15*) and placed in a cabinet containing the solvent mixture. One hour was allowed for equilibration of the paper with the solvent-vapor atmosphere, then solvent mixture was added to the trough and the cabinet was sealed with tape. After development the strips were air-dried, heated for 20 minutes at 85° C., then sprayed with 0.2% ninhydrin in 1-butanol which was saturated with water, air-dried, heated for 5 minutes at 70° to 75°, and then examined. Each kind of paper (with the exception of S.S 589 Red Ribbon) was run at least twice with each solvent.

RESULTS AND DISCUSSION

The following factors were considered in analyzing the data and evaluating the papers. The behavior of each strip was graded on a scale of 4:

Degree and clarity of separation of amino acid spots

Diffuseness of the spots

Degree of formation of "brown front"—that is, yellow, brown, or green stains or streaks, presumably caused by impurities in the paper, which accompany the solvent front down the strip

Extent of formation of "tails" and "beards" (*5*) on amino acid spots—that is, zones trailing out behind or running on ahead of the spots and giving color reactions with ninhydrin noticeably different from those of the spots

Swerving of the spots—that is, deviation from vertical development

Rate of movement of solvent down the strip

With three exceptions—R.A. 202, 226, and 230—all the papers tested appear to be suitable, but to different degrees, for separating the amino acid mixtures. These papers gave a high flow rate of developer, but the zones of amino acid were diffuse spots and streaks, and showed poor separation under the conditions of the test.

The papers which gave best results, all factors considered, were, in descending order of excellence for a given solvent:

Collidine: W. 3, S.S. 595, W. 4, W. 1
Phenol: W. 3, S.S. 595, W. 1, W. 4
1-Butanol: S.S. 589 Black Ribbon, S.S. 595, S.S. 598, W. 3
2-Butanol–formic acid: W. 3, W. 1, W. 2, S.S. 602
2-Butanol–ammonia: S.S. 589 Blue Ribbon, Red Ribbon, White Ribbon, W. 1

The differences between the papers listed above for each solvent were relatively slight, and the grading of factors in the six categories listed could not be as objective as might be wished. Even

when different papers were run at the same time in a cabinet with the same amino acid mixture and the same solvent, the R_F values for given amino acids often differed widely among the papers. An example of the data is given in Figure 1. These differences may be caused by differences in adsorption of the amino acids by the different papers, in extent of adsorption of the phases by the paper supports, in kind or amount of impurities in the papers, or in the closeness of approach to equilibrium in the distribution of the amino acid between the phases, which might be related to the rate of flow of solvent. A combination of these effects may operate. In any case it would appear that with most of these papers the separations are not described by a simple liquid-liquid distribution.

Taking paper W. 1 as a standard [which is reasonable because Consden, Gordon, and Martin (*5*) found the R_F values of selected amino acids to be close to the theory for simple liquid-liquid distribution], a group of five papers showed rather interesting differences from the standard behavior. These papers—S.S. 507, 576, 589 Black, 589 Blue, and 589 White Ribbon—showed a circular aspartic acid spot in collidine, running with the same R_F value as histidine. With the same mixture of amino acids, all the other papers exhibited the usual elongated aspartic acid spot (*6*) running well behind histidine. Whereas with most of the other papers the amino acid spots were of the usual purple to red-purple color (*5*), with these five papers the colors were blue to blue-purple. These peculiarities were unaffected by the use or nonuse of ammonia vapor when the amino acid mixtures were applied to the paper.

With phenol these same five papers showed higher than normal R_F values for aspartic acid and greatly lowered values for arginine. On S.S. 507 and 576, aspartic acid and arginine move as circular spots, whereas both amino acids tend to move as elongated spots, or streaks, on most of the other papers. When ammonia vapor was omitted during application of amino acid mixtures to papers S.S. 507, 576, and 589 Blue Ribbon, the first two papers gave normal R_F values for aspartic and the R_F values for arginine were lowered still further. The behavior of 589 Blue Ribbon was not changed. The ninhydrin colors of the spots were again blue to blue-purple with these papers.

With the remaining three solvents the five papers showed no unusual behavior, though the colors with ninhydrin were still on the blue side.

According to the manufacturers (*10*), these five papers are an especially purified grade. Presumably certain salts (or other impurities) have been removed from these papers in their purification, for they gave little or no bearding of spots. Bearding has been attributed to the action of copper ion in the paper (*5*). The absence of bearding should make these papers especially useful for quantitative analysis (*1–3, 7, 8, 11–13, 15, 16*).

Of the five papers mentioned above, S.S. 507 is probably the best for quantitative use, though the rate of movement of solvent in this paper is very slow. As a generally useful paper W. 3 may be recommended for being a thick, absorbent paper which gives sharply resolved spots. Paper S.S. 595 has the merit of giving excellent separation with generally higher R_F values than given on W. 3. Where the desire for speed of analysis outweighs considerations of sharp separation and resolution of spots, papers S.S. 604, 598, and 589 Black Ribbon, over which the solvent moves 40 cm. in about 6 to 8 hours, may be found useful.

Other observations, together with the results of studies of other papers and varied conditions, will be forthcoming when present work is completed. It is conceivable that papers found useful in the separation of amino acids may not be the same as those found useful for other separations. The implications of this investigation are therefore explicitly limited to the stated systems.

SUPPLEMENTARY TESTS

Fifty-three industrial papers from the Eaton-Dikeman Company were tested against the phenol and collidine solutions described above, using the rapid method of Rockland and Dunn (*14*). The papers could be divided into the groups listed in Table I. Streaking of spots and poor separations occurred with the papers in Group A. Papers in Group B varied among themselves, but were better than the Group A papers. The first 16 papers listed in Group B were tested in phenol and collidine, using the procedure described above. The remaining papers in Group B were plainly of limited utility for this work—for example, a gray paper (622 Gray) would mask the normally gray-colored ninhydrin spots, as of histidine. These 16 papers were compared with Whatman No. 1, and none were found superior to this paper. Papers 7 Purity, 612, 613, 622 White, and 954 showed a speeded up aspartic acid spot in collidine and phenol and a retarded arginine spot in phenol, a phenomenon similar to that reported above for papers 507, 576, etc.

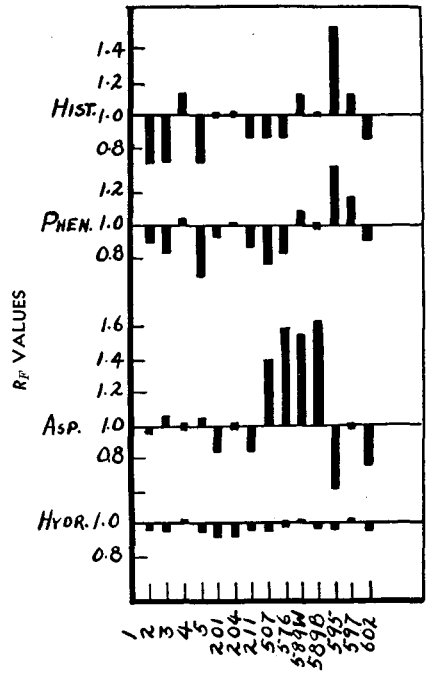

Figure 1. Relative R_F Values of Amino Acids

R_F values for four amino acids, two each in two different solvents, are shown for fifteen filter papers. In each group the papers were run at the same time in the same cabinet, and the same mixture of amino acids was used with each paper. The R_F values are plotted on the ordinate relative to Whatman No. 1, which is taken as the norm. Hist., histidine in 1-butanol–ammonia; Phen., phenylalanine in 1-butanol–ammonia; Asp., aspartic acid in phenol; Hydr., hydroxyproline in phenol. On the abscissa are listed the filter papers; W refers to White Ribbon, B to Blue Ribbon. The first five are Whatman papers; the next three are Reeve Angel; the rest are Schleicher and Schuell papers.

Table I. Enumeration of Eaton-Dikeman Company Filter Papers

Group A		Group B	
215	625.030	1 Purity	048
255	627.023	4 Purity	620
301.030	627.026	5 Purity	622 Gray
301.050	627.030	7 Purity	629
320	628.026	248	655
614	628.035	604	
615	632	607	
616	633.70	608	
617	633.75	609	
619	634	611	
623.026	637	612	
623.030	638	613	
623.038	640	622 White	
624.022	641	950	
624.035	648	952	
625.026	652.050	954	

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Determination of Traces of Sulfur in Organic Compounds

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Improvements in the method for the determination of 1 to 200 p.p.m. of sulfur in organic compounds by combustion with excess air in a heated tube have provided a satisfactory means for the analysis of solids and liquids which are difficult to analyze by conventional methods. The principle of the method is well established, but the design of an atomizer to deliver the sample as a fine spray in a stream of air to the combustion zone at an easily controlled rate, and improvements in the absorption of sulfur dioxide and nephelometric determination of sulfur as barium sulfate now permit wider application of this principle. Better absorption of oxides of sulfur has been obtained by condensing water from the combustion in a chilled trap containing sodium perborate solution before passing the gases through absorbers. Precipitation from ammoniacal solution, in the presence of gum arabic as a stabilizer, improved the stability of the barium sulfate colloid. The method has been successfully applied to liquids such as cetane, decahydronaphthalene, *o*-nitroanisole, benzaldehyde, xylidine, and diethylaniline, and solutions of solids, such as naphthalene, dinitrobenzene, nitronaphthalene, and *p*-nitrophenetol. Accuracy of the order of 5 to 10% of the amount of sulfur present and a precision of ± 2 parts in the range 2 to 100 p.p.m. of sulfur have been attained with both volatile and nonvolatile sulfur sources.

SEVERAL reliable and accurate methods for the determination of sulfur in organic compounds by combustion have been widely used. However, these techniques are not applicable to the determination of total sulfur in the range 1 to 200 p.p.m. where relatively nonvolatile organic compounds, or solutions containing high concentration of solids, are involved. Briefly, these methods may be classified as:

The Parr bomb method, in which sulfur in organic compounds is converted by oxidation with sodium peroxide or oxygen under pressure to sodium sulfate and precipitated as barium sulfate, is well known for accuracy and reproducibility (*17*). However, the practical limits of this procedure are restricted by the sample size to sulfur content greater than 0.1%.

The A.S.T.M. lamp method (*1*) has been considered reliable for the estimation of total sulfur in volatile petroleum products in concentrations, in general, greater than 20 p.p.m. Considerable attention has been given to improvements in burner and absorber design, and the method has been adapted to a variety of relatively volatile liquid products (*3–7, 9, 15, 16, 18, 19*). Although the lower limit of this procedure has been extended to 2 p.p.m. of sulfur (*22*), the method fails if volatility or flammability is low, large amounts of solids are in solution, or the sulfur is in a form that does not volatilize from the wick into the flame.

Combustion, in oxygen, of sulfur-bearing samples from boats in horizontal tube furnaces, has been well established for concentrations down to 0.1% (*1, 12–14, 20*). However, limitations on sample size do not permit assay for sulfur in the range 1 to 200 p.p.m.

Oxidation, of the sulfur in organic derivatives with nitric acid in a sealed tube following the principles originated by Carius has been well developed within its obvious limitations of sample size and manipulative difficulties (*2–11*). Successful application to the sulfur range under discussion has not been attained.

Sulfur has been determined with accuracy in a wide range of organic liquids by allowing a sample to drop slowly in an air stream into a vertical, packed tube mounted in a furnace. The gaseous oxidation products are drawn by vacuum from the lower end through hydrogen peroxide to absorb sulfur oxides (*10*). Although combustion of small samples containing relatively large percentages of sulfur appears to yield excellent results, in the authors' experience application to large samples for estimation of traces is not satisfactory. Complete and smooth combustion has not been obtained unless the sample is atomized into exceedingly small droplets that approach an aerosol in character.

A novel procedure in which the sulfur in organic compounds is quantitatively converted to hydrogen sulfide by passing a sample with hydrogen over alumina at 900° C. illustrates an entirely different approach to this problem (*8*). Although the detection of sulfur as sulfide has valuable applications, the method involves the use of hazardous hydrogen not readily available in many laboratories, uncertainty with respect to application to all types of sulfur compounds, development of a specialized technique, and known absorption of variable quantities of sulfur in the alumina catalyst.

The method presented in this paper overcomes many of these difficulties by introducing the sample, either a liquid or a solution of a solid in an appropriate solvent, into a combustion tube in the form of a fine spray in a stream of excess air. The vital factor is the design of the atomizer, which permits precise control of the feed and air delivery rates to the combustion zone. The gaseous oxidation products are chilled and the sulfur oxides are converted to sodium sulfate by absorption in dilute aqueous sodium perborate solution. The carbon dioxide is expelled by heating the absorber solution to boiling and acidifying with nitric acid. After cooling, excess ammonium hydroxide and a protective colloid are added. This sample is divided into two aliquots,

and crystalline barium chloride is dissolved in one. Both aliquots are then acidified with nitric acid, the amount of colloidal barium sulfate is determined by nephelometric procedure with the barium-free aliquot as reference, and the total weight of sulfur is read from a calibration graph.

APPARATUS

Assembly of the apparatus is shown schematically in Figure 1.

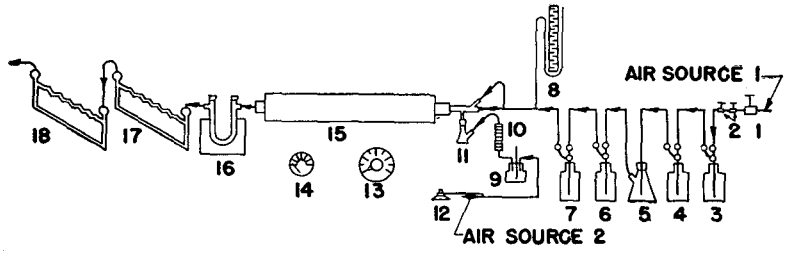

Figure 1. Flow Diagram of Sulfur Combustion Microdetermination

1. Needle valve
2. Air reducing valves
3. Empty trap
4. Sulfuric acid trap
5. Asbestos-filled trap
6. Water trap
7. Sodium hydroxide trap
8. Manometer (Nujol)
9. Mercury safety trap
10. Calcium chloride drying tube
11. Spray-type burner assembly (Figure 2)
12. Feed regulator
13. Variac, 2 kw.
14. Voltmeter
15. Combustion tube
16. Cold trap in ice bath
17. No. 1 circulation gas absorber
18. No. 2 circulation gas absorber

The spray-type burner assembly, 11 (Figure 1), consists of the atomizer, *B*, and sample holder, *A*. The sample is forced into the orifice by slight air pressure at *A*-1 and the spray is controlled by the adjustable sleeve, *B*-3 (fabricated by Ace Glass, Inc., Vineland, N. J., Catalog No. AL-91).

The combustion tube, 15, is a 1 × 48 inch quartz tube wrapped to within 2 inches of either end with 1-inch (2.5-cm.) asbestos tape. This forms the foundation for 22 feet of No. 19 V-Chrome resistance wire (0.518 ohm per foot) wrapped approximately 1 turn per inch. A second layer of asbestos tape serves to insulate and hold the wire in place. Thermal insulation is provided by placing the assembly in a 46-inch piece of magnesia pipe lagging.

The gas absorber train, 16–18 is assembled from a cold trap, 16 (Ace Glass Catalog No. AL-88), and two circulating gas absorbers (Ace Glass Catalog No. AL-74) connected in series.

The Cenco-Sheard-Sanford photelometer (Catalog No. 12335) is equipped with the blue filter (Catalog No. 873094). A standard reference curve for relating sulfur content to light absorption is plotted from photelometer readings of water solution containing known amounts of sulfur (C.P. sodium sulfate) precipitated as colloidal barium sulfate as outlined under procedure.

REAGENTS

Sodium perborate, 1% solution in distilled water.

Dilute nitric acid, 20 ml. of concentrated C.P. nitric acid diluted to 100 ml. with distilled water.

Dilute ammonium hydroxide, 30 ml. of concentrated C.P. ammonium hydroxide diluted to 100 ml. with distilled water.

Gum arabic, 10 grams of U.S.P. gum arabic dissolved in 100 ml. of distilled water by heating on a steam bath and filtering to remove sediment.

Barium chloride, C.P. reagent crystals.

PROCEDURE

Preparation of Sample. Liquid samples that burn or feed with difficulty and solid materials are dissolved in a suitable solvent of known sulfur content. Oxygenated solvents such as alcohols, ethylene glycol ethers, dioxane, or dimethyl formamide are preferred. Correction for the sulfur introduced with the solvent is then made in the final calculation.

Operating Details. The combustion train is assembled as shown in Figure 1. The air feed scrubber line is prepared as follows:

1, 2. Connect air reducing valves as shown.

3. Assemble empty to serve as a safety trap.

4. Add 96% sulfuric acid to 2-inch inlet tube immersion level for removal of organic and other foreign materials.

5. Pack with asbestos fiber to remove sulfuric acid mist.

6. Add sufficient distilled water to obtain 2-inch inlet tube immersion to replace the water vapor in the air stream and avoid evaporation in the following scrubber.

7. Add sufficient 30% by weight sodium hydroxide solution for 2-inch inlet tube immersion to remove sulfur compounds.

Make connections wherever possible with Tygon tubing which contains no sulfur. Place just sufficient mercury in safety trap 9 to form a seal that permits application of slight air pressure to the sample flask, *A*, of the atomizer assembly, 11. Pack drying tube 10 with alternate layers of calcium chloride and indicating Drierite to assure dry air over the sample. Connect the burner to the combustion tube in furnace 15 by wrapping sleeve *B*-3 (Figure 2) with sufficient asbestos paper to form a stopper-tight fit when pressed into place. Connect the cold trap, 16, to the other end of the combustion tube in a similar fashion.

Replace the sample flask of unit 11 with a test tube and, while running a slow stream of air through the scrubber train and combustion zone, heat the combustion tube slowly to dull red heat (800° to 900° C.) over a 1-hour period. Power input at 100 volts has been found optimum for the operation of a furnace built according to the specifications outlined.

Measure out 60 ml. of 1% sodium perborate reagent and add a quantity (approximately 5 ml.) sufficient to form a liquid seal in cold trap 16. Aliquot the remainder into the circulating gas absorbers, 17 and 18. Pack the cold trap, 16, in ice and connect the members of the scrubber unit, 16–18, as shown in Figure 1.

Place 20 to 30 grams of sample in the sample holder, *A*, of unit 11, and record the gross weight to the nearest 0.01 gram. Replace the test tube installed for the preliminary air sweep, and connect the drying tube, 10, to the side arm, *A*-1, with valve 12 open.

Adjust the air flow (No. 1 air source) to a rate that will produce a differential pressure (mineral oil) in the manometer, 8, equivalent to a flow rate of 1.5 to 2.0 liters of air per minute. (This is an average value which will vary in accordance with the oxygen requirements of the particular sample tested.) Slowly close valve 12 until liquid is delivered to the orifice of the atomizer, 11, and

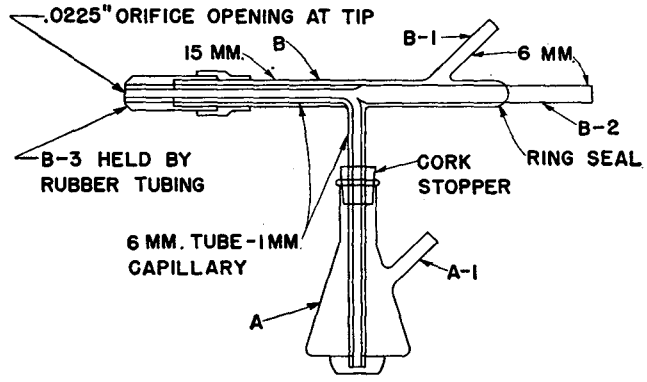

Figure 2. Spray-Type Burner

A. Sample holder
A-1. Air inlet to feed sample
B. Atomizer
B-1. Excess air feed
B-2. Low pressure air
B-3. Adjustable sleeve

adjust the position of the orifice relative to the port in sleeve *B*-3 by sliding in or out slightly until a fine spray is generated. This adjustment varies only slightly from sample to sample. The sample feeding may be stopped instantly at any time by opening valve 12. Good results are obtained at a feed rate of 10 grams per hour, and a total sample weight of 20 to 25 grams is recommended for materials containing less than 5 p.p.m. of sulfur.

When sufficient sample has been burned, replace sample holder *A* with a test tube and sweep the assembly with air at the burning rate for 30 minutes to assure complete removal of sample, and complete absorption of the sulfur oxides.

Record the gross weight of *A* and residual sample to the nearest 0.01 gram.

Close valve 1, and combine the scrubber solutions and washings from cold trap 16 and scrubbers 17 and 18 in a 250-ml. beaker.

Add 0.2 gram of solid sodium perborate, cover with a watch glass, and heat to boiling. Add 2 ml. (graduated pipet) of dilute nitric acid, and check acidity. If not definitely acid to Congo red, add 1 ml. of acid.

Boil down to a small volume (less than 40 ml.), cool to room temperature, add 1 ml. of dilute ammonium hydroxide solution, and check alkalinity. If not definitely alkaline to Brilliant Yellow, add 1 ml. more of ammonium hydroxide reagent. Add 5 ml. of 10% gum arabic solution and filter into a 50-ml. volumetric flask (No. 1 Whatman filter paper) if the solution contains more than a trace of sediment. Rinse the beaker with distilled water into the filter, or directly into the flask if filtration was unnecessary, and dilute to volume with distilled water. After thorough mixing, transfer one half (25-ml. graduated cylinder) back into the beaker, add 0.5 gram of barium chloride, and dissolve by swirling. Add 0.5 ml. (graduated pipet) of dilute nitric acid to each aliquot and check acidity. Add 0.5-ml. aliquots of acid if not definitely acid to Congo red.

With the barium-free aliquot as reference and the blue filter in place, determine the light transmittancy of the barium sulfate colloid in a 1-cm. cell in a photelometer. Read the total grams of sulfur from a standardization curve. Subtract from this value the reagent blank determined by duplicating the above manipulation with a test tube in place of the sample holder, *A*, and sulfur introduced in any solvent employed. When a blank determination over a 2-hour scrubbing period shows a sulfur content higher than 1.5 p.p.m., renew all liquids in the air scrubber bottles and check each of the reagents for contamination.

Calculation.

$$\frac{(\text{Total grams of sulfur} - \text{grams of sulfur in blank}) \times 1{,}000{,}000}{\text{weight of sample burned}} = \text{p.p.m. sulfur}$$

DISCUSSION

Several burners of radical design were constructed, tested, and found unsatisfactory. The wick-type burners were not found suitable for viscous or low vapor pressure liquids. Drip-type burners, with or without preheating or prevaporizing chambers produced irregular feed, carbonized at the feed orifice and required constant adjustment. A specially constructed aerosol generating aspirator produced preferential evaporation of the solvent.

Complete combustion of any organic compound for analytical purposes requires sufficient oxygen which varies somewhat with the structure and with oxygen and hydrogen content of the compound under consideration. It is important, therefore, to have a sufficient excess of air at a constant rate of supply, and properly scrubbed to remove sulfur impurities. The use of concentrated sulfuric acid and sodium hydroxide scrubbers has been found to be convenient and adequate for 50 to 60 hours' operation with ordinary laboratory compressed air sources. Because blank determinations are considered necessary for accuracy, the use of a preburner recommended by several workers was not considered justified.

The combustion of organic derivatives deficient in hydrogen or oxygen is often incomplete unless an oxygenated solvent, such as alcohol, is used as a diluent (Table I). The use of solvents also aids in the combustion of viscous, low vapor pressure, and high flash point liquids, as well as furnishing a medium through which solid samples can be analyzed (see Table II, *p*-nitrophenetol, and Table I, 17th, 22nd, 24th, 25th, 26th, and 28th, items).

Experience has shown that it is necessary to condense, by cooling, the water produced by combustion in order to absorb the sulfur dioxide more efficiently. Furthermore, two gas circulating absorbers are necessary to obtain complete recovery of sulfur at burning rate suitable for routine analysis. The choice of sodium perborate as the absorbing oxidant was a matter of expediency. Samples available in the laboratory were very nearly sulfur-free, and the relatively high alkalinity of this reagent appeared to be desirable. However, other oxidizing reagents, such as hydrogen peroxide, potassium iodate, and sodium hypobromite, have been recommended (*5*, *10*, *13*, *21*).

The use of a protective colloid when precipitating barium sulfate has been found necessary. The stability of this colloidal precipitate is considerably improved by precipitating alkaline. Gum arabic was found to be the best of a number of protective agents tested. The light transmittancy of the barium sulfate colloid produced from burning 9 to 13 grams of sample containing 105 p.p.m. of sulfur did not change sufficiently over a 45-minute period to give more than 0.5% difference in photelometer readings. Furthermore, sufficient stability to permit determination of 190 to 200 p.p.m. of sulfur (Table III) has been attained. Satisfactory results were not obtained when determining the sul-

Table I. Examples of Application

Material	Sample Weight, G.		Solvent	Sulfur, P.P.M.	
n-Heptane	12.86	9.16	None	32	38
n-Heptane	8.16	4.42	None	13.4	11.3
Iso-octane	6.89	12.99	None	39	42
Iso-octane	10.26	8.12	None	1.0	1.1
Gasoline 1	10.70	7.49	None	24	28
Gasoline 2	17.99	6.69	None	20	20
Cetane	3.32	6.67	None	3.6	3.5
Diisobutylene	11.19	9.58	None	60	60
Cyclohexane	13.22	15.76	None	22	23
Cyclohexane	3.41	2.66	None	5.8	7.5
Pinene	4.17	5.85	None	31	32
Pinene	4.44	5.41	None	74	77
Decahydronaphthalene	4.54	5.84	None	Less than 1	
Tetrahydronaphthalene	1.49	2.43	None	20	21
Benzene, commercial	13.92	4.48	None	15.1	15.5
Toluene	8.18	5.83	None	37	38
Naphthalene	7.78	5.21	Benzene	4	5
Nitrobenzene	11.83	5.48	Methanol	6.8	5.5
Nitrobenzene	3.52	2.77	None	3.0	3.6
o-Nitrotoluene	5.72	5.23	Methanol	5.4	7.7
o-Nitrotoluene	2.54	2.62	None	3.9	3.8
m-Dinitrobenzene	2.90	4.20	Dioxane	16	16
m-Dinitrobenzene	1.54	1.51	Dimethyl formamide	45	46
Nitronaphthalene	5.38	6.02	Dioxane	21	18
o-Nitroanisole	1.71		Ethyl alcohol	18	..
	6.80	3.09	Dioxane	18	19
p-Nitrophenetole	1.61	..	Dimethyl formamide	198	..
Benzaldehyde	8.51	7.80	Ethyl alcohol	4.7	5.0
Dimethyl formamide	6.54	1.75	None	12	11
o-Toluidine	5.12	..	Methanol	6	..
Xylidine	5.74	..	Benzene	7	..
Diethylaniline	2.12	2.67	None	14	15
Diethylaniline	2.31	1.42	None	13	14

Table II. Determination of Precision

Trial No.	Sample Size, G.	Feed Rate, G./Hour	Sulfur Found, P.P.M.	Deviation from Mean, P.P.M.
Sulfur in Ethyl Alcohol (2B Alcohol)				
1	9.71	5.55	13	2.8
2	8.96	5.97	17	1.2
3	8.19	7.80	17	1.2
4	6.94	4.63	17	1.2
5	9.33	4.67	13	2.8
6	8.36	5.57	17	1.2
7	6.75	3.69	16	0.2
8	4.71	3.14	16	0.2
9	5.59	3.73	17	1.2
10	7.13	3.57	15	0.8
Av.	7.57	4.83	15.8	±1.3
Standard deviation				±1.6
Sulfur in *p*-Nitrophenetole-Dioxane Solvent (1 to 2 Parts of Solvent/Part of Sample)				
1	5.01	1.66	12	1.2
2	3.83	0.69	15	1.8
3	7.12	1.09	14	0.8
4	4.38	1.26	15	1.8
5	4.88	0.68	12	1.2
6	5.62	0.77	11	2.2
Av.	5.14	1.03	13.2	±1.5
Standard deviation				±1.7

Table III. Determination of Accuracy

Trial No.	Sample Size, G.	Burning Time, Hours	Sulfur, P.P.M. Added[a]	Found
		Thiophene in Benzene		
1	12.50	2.3	None	4.8
2	12.25	2	None	6.5
3	14.15	2.7	10	9.2
4	12.55	2.3	10	10.4
5	12.95	2	16.5	18.5
6	12.80	2	106	104
7	9.45	1.5	105	103
		Tetramethylthiuram Monosulfide in Benzene ($AlCl_3$ Treated)		
1	11.42	1.5	None	1
2	12.92	2.5	5.6	5.4
3	9.71	2.5	5.6	6.1
4	6.86	1.5	5.6	5.8
5	11.84	1.5	5.6	6.0
Av.	10.55	1.9		5.8 ± 0.3
1	19.39	3.5	None	2
2	20.26	3.3	6.6	6.5
3	10.63	3	6.6	7.0
4	9.21	2.8	6.6	7.6
Av.	14.88	3.15		7.0 ± 0.5
		Tetramethylthiuram Monosulfide in Dioxane		
1	9.86	2	None	2
2	15.29	1.8	None	2
3	9.70	2	20	20
4	9.65	1.8	20	22
5	12.67	2	20	17
6	6.66	1.5	20	18
7	9.53	1.5	20	19
8	12.29	1.5	20	21
9	19.40	2	20	19
10	11.67	1.5	20	20
Av.	11.65	1.8		19.5 ± 1.5

[a] "Added" includes initial sulfur in solvent.

fur content of samples containing more than 1 or 2% halides, but work is in progress on a solution of this problem by introducing a variation in the absorption technique.

CONCLUSIONS

A satisfactory method has been developed for the accurate determination of microquantities of sulfur in organic compounds. The principle of the method is not new, but the burner design developed to feed the sample to the combustion zone, and the method of sample preparation, now permit wide application of this principle. Accuracy of the order of 5 to 10% of the amount of sulfur present, and a precision within ±2 p.p.m. in the range 2 to 100 p.p.m. of sulfur has been attained.

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Quantitative Fluorometric Microdetermination of Alloxan Monohydrate as Riboflavin

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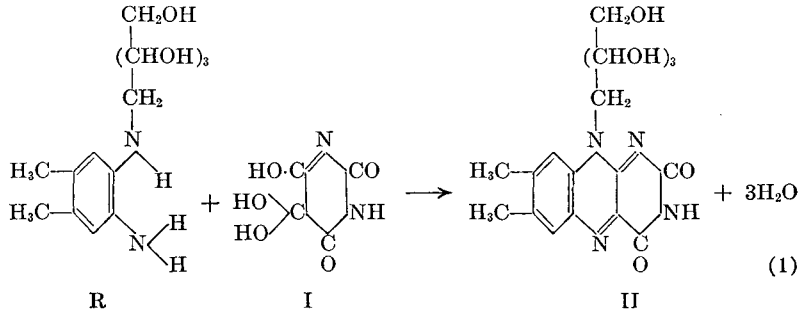
IN THE course of work on alloxan monohydrate (*19*, *21–23*), the authors had occasion to ascertain the concentration of very dilute solutions of the compound and to determine the purity of commercial samples. One method tried was that outlined in a note on "preliminary studies leading to a sensitive and specific test for the quantitative estimation of alloxan in pure solution" (*2*). The procedure involves the condensation (*5*, *9*, *13*) of alloxan monohydrate (I) with D-1-ribitylamino-2-amino-4,5-dimethylbenzene (R) to yield riboflavin (II) as shown.

This reaction had been carried out, in ca. 67% acetic acid, at 100° for 3 hours. A huge excess of R, amounting to some 1185 to 11,850 times the theoretical quantity, was employed, presumably with the object of driving the reaction to completion. Because riboflavin, when activated by light of certain wave lengths (*8*, *12*), gives off a yellow-green fluorescence whose intensity is directly related to its concentration at any given pH, the amount of riboflavin produced in accordance with Equation 1 may be determined by ob-



R I II (1)

A quantitative fluorometric method for the determination of alloxan monohydrate in solution is based upon the previously reported formation of riboflavin on condensation with D-1-ribitylamino-2-amino-4,5-dimethylbenzene, even at extreme dilutions. A shortened procedure may be employed, provided that the results then obtained are multiplied by a factor. The purities of some commercial samples of alloxan monohydrate have been determined.

servation of the resulting fluorescence in the riboflavin range, thus giving a measure of the amount of alloxan monohydrate present, provided that R itself displays no fluorescence in this range. If, however, R is or becomes fluorescent, it was apparently implied that the amount of R consumed by Reaction 1 is negligible compared to the excess of R present, and that the fluorescence contributed by R may be subtracted from the total fluorescence to give that due to the riboflavin formed.

DISCUSSION OF RESULTS

Before testing this procedure, a fluorometer was calibrated by means of various known dilutions of a standard, unheated riboflavin solution, brought to appropriate pH (see Figure 5). The method for alloxan determination was now applied exactly as previously described (*2*). It was found that D-1-ribitylamino-2-amino-4,5-dimethylbenzene itself displayed fluorescence and that portions of the chosen sample of alloxan monohydrate invariably gave rise to much less than the theoretical amount of riboflavin. Control experiments with pure riboflavin solutions treated in the same manner showed that these low values were partly ascribable to the use of ordinary borosilicate glass test tubes [as recommended (*2*)], which permitted ingress of light of wave lengths deleterious to riboflavin. Encasement of the tubes with tin foil, or use of Lifetime Red test tubes, prevented destruction of riboflavin by light. Given concentrations of riboflavin then elicited the same fluorescent response, regardless of whether the solutions were preserved at room temperature or at 100° for 3 hours under laboratory lighting.

A second modification consisted in the use of the monohydrochloride of D-1-ribitylamino-2-amino-4,5-dimethylbenzene instead of the free base; the latter, even as crystals, quickly develops color and fluorescence on storage in an amber bottle, giving rise to a large "blank" reading. The authors found that the dry, crystalline hydrochloride is very stable and that its fresh solution in glacial acetic acid displays a low fluorescence.

Repetition of the alloxan determinations by condensation at 100° with D-1-ribitylamino-2-amino-4,5-dimethylbenzene hydrochloride in red test tubes gave results which, although higher, were still only approximately 15% of the theoretical (see Figure 1). This could be tentatively interpreted as suggesting either that the alloxan monohydrate sample was grossly impure or that the reaction shown in Equation 2 did not proceed to completion under the given conditions.

$$R.HCl + I \longrightarrow II + HCl + 3H_2O \quad (2)$$

The latter explanation might appear improbable because of the enormous excess of R.HCl employed, amounting to some 500 to 5000 times that required by Equation 2; but it is well known that if reaction 1 is conducted on a macro scale, even at high concentrations with an excess of alloxan monohydrate, the yield of riboflavin isolated amounts to only some 5 to 10% of the theoretical (*5*, *9*, *13*).

If, however, boric acid is added to the reaction solution, the yield of riboflavin isolated in macro experiments is greatly increased (*7*, *10*, *11*, *14–16*), reaching some 95% in anhydrous solutions. The authors therefore modified the original conditions for microdetermination (*2*) by the introduction of boric acid into the reaction mixture, thereby increasing the yield of riboflavin at 100° to ca. 57% of the theoretical (see Figure 1) and extending to a much lower limit the dilution at which the alloxan monohydrate was determinable. Indeed, the "sensitivity" of the determination with boric acid was more than 3.5 times that without it. The reaction in presence of boric acid was now repeated for various time intervals, and it was found that heating for more than 1 hour was unnecessary (see Figure 2).

From the foregoing, it appeared likely that the low results obtained at 100° might be attributable to one or more of three reactions competing with that shown in Equation 2—viz., decomposition of I or of R.HCl (and, perhaps, of II) in the medium at 100° C. It was found that I does not decompose appreciably on heating in the reaction medium at 100° for 1 hour. Similarly, II formed from alloxan monohydrate at room temperature during 3 days does not decompose when the reaction solution is heated in a red tube at 100° for 1 hour. On the other hand, a glacial acetic acid solution of R.HCl decomposes at room temperature as measured by reactivity towards alloxan monohydrate solution (see Figure 3), and R.HCl decomposes in the hot reaction medium, as shown by the change in fluorescence of the "blank" (see Figure 2).

Because of the latter observation, it was decided to ascertain the effect of conducting the condensation (Equation 2) at room temperature. After 72 hours, the yield of riboflavin obtained was ca. 80% of the theoretical (see Figure 2). Hence, the alloxan monohydrate sample contained 20% of impurity, it had undergone decomposition to this extent during the 3 days at room temperature, or Reaction 2 had not gone to completion. Now, macro experiments on reduction to alloxantin dihydrate revealed that the purity of the alloxan monohydrate sample used was at least 96%. Secondly, it was found that decomposition of alloxan monohydrate (1.0 microgram per 3 ml.) in the reaction medium

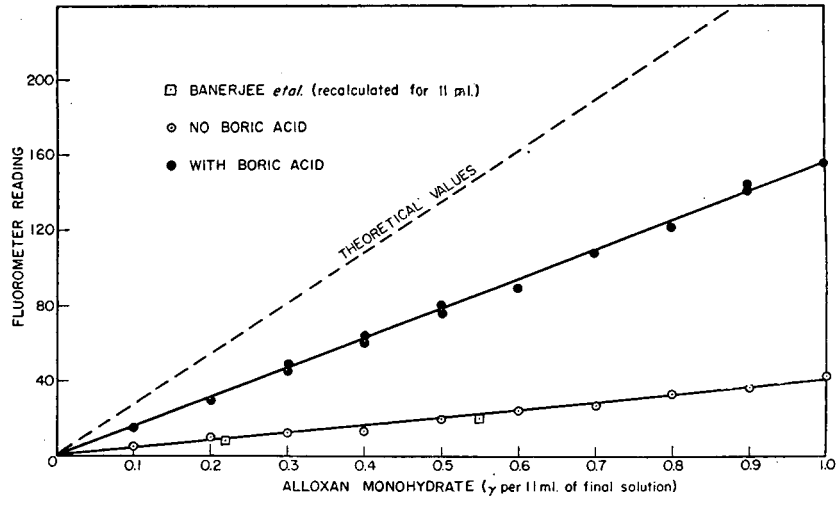


Figure 1. Formation of Riboflavin from Alloxan Monohydrate during 3 Hours at 100° C.

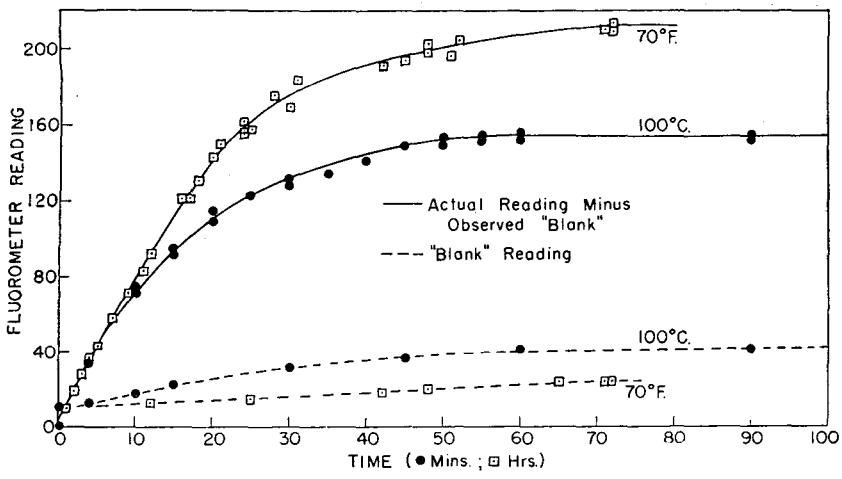


Figure 2. Rate of Formation of Riboflavin from Alloxan Monohydrate

In presence of boric acid at 100° C. and 70° F.

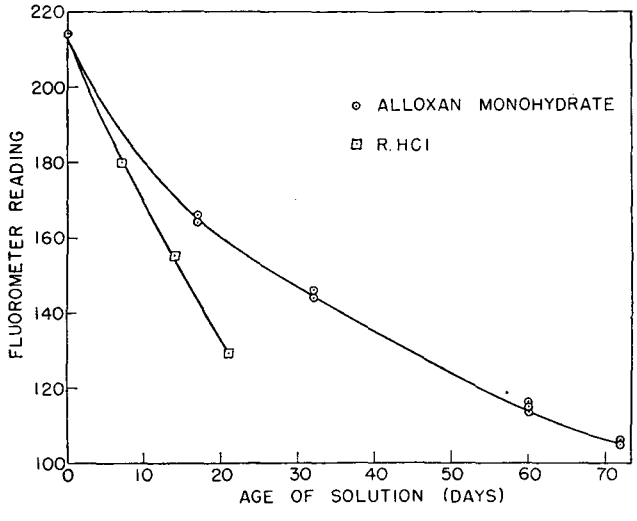


Figure 3. Rate of Decomposition of Alloxan Monohydrate and R.HCl at Room Temperature

Alloxan monohydrate, 1 microgram per ml., solution B; R.HCl in glacial acetic acid

(1.5% boric acid in ca. 67% acetic acid) does not occur appreciably during 3 days at room temperature [although, as shown in Figure 3, decomposition of alloxan monohydrate in the stock solution (1.0 microgram per ml., 4.5% boric acid in 5% acetic acid) takes place slowly at room temperature (time to half value, about 72 days)]. Thirdly, by increasing the reaction time to 6 days, the yield was only raised to 87% of the theoretical.

Consequently, experiments were now conducted for 72 hours at room temperature with various proportions of fresh R.HCl solution. The resulting asymmetric sigmoid (see Figure 4) indicated that the yield of riboflavin from 1.0 microgram of alloxan monohydrate is increased to 95% of the theoretical by doubling the proportion of R.HCl solution hitherto employed. Furthermore, under these conditions, 0.25 ml. of R.HCl solution itself gives rise to the same amount of fluorescence as is produced when 1.0 microgram of alloxan monohydrate is present. Consequently, the whole of the "blank" value should not be subtracted. Correcting for this effect, the yield of riboflavin obtained from 1.0 microgram of alloxan monohydrate plus 2 proportions of R.HCl during 3 days at room temperature is, within the limits of experimental error, practically quantitative. This shows that the alloxan monohydrate sample selected was extremely pure. The fluorometer was therefore calibrated in terms of alloxan monohydrate under these conditions (see Figure 5); the "sensitivity" was about 6 times that of the original method (*2*). If the original method (*2*) were applied to the estimation of alloxan monohydrate in biological fluids without allowance for the low yield of riboflavin thereby resulting, the results obtained would indicate only ca. 15% of the alloxan monohydrate actually present, and low concentrations might well escape detection.

Repetition of the reaction at 100° C. for 1 hour, using a double proportion of R.HCl solution, gave results which, uncorrected, were about 83% of the theoretical (see Figure 5). Owing to the low solubility of R.HCl in glacial acetic acid, further increase of the R.HCl concentration in the reaction solution was not feasible without further changing the conditions originally specified. However, if the values found for 1 hour's treatment (at 100° in the presence of boric acid and 2 proportions of R.HCl) are multiplied by a factor of 1.20, the true values are obtained. The shorter procedure may then be employed for routine determinations.

APPARATUS

A Coleman photofluorometer (Model 12 A) was used in conjunction with filter B-2 (to pass the 436 mμ mercury band), filter PC-2 (to screen the phototube from that wave band), and a constant voltage transformer (Sola). The maximum reading on the fluorometer was 100 scale divisions. Before routine use of the instrument, the output voltage of the constant voltage transformer (connected between the supply voltage and the autotransformer) was measured after the mercury vapor lamp had reached proper operating intensity. The appropriate input terminal was then selected on the autotransformer. The instrument gave a linear response (*17*) to different concentrations of riboflavin in the range employed (see Figure 5). Readings were reproducible only to within ±1 scale division; hence the actual error was correspondingly greater for those solutions which had to be diluted to bring the fluorescence intensity within the range of the instrument. Only the first reading made on a cuvetteful of any solution containing riboflavin was significant; each exposure to the ultraviolet light of the fluorometer resulted in a diminution of the reading.

Standard borosilicate glass test tubes (18 × 150 mm., with rim) were used in certain experiments noted. In all other experiments, "low actinic" Lifetime Red Pyrex test tubes (manufactured by the Corning Glass Works, Corning, N. Y.) of the same dimensions were employed. They were labeled with test tube markers (Fisher Scientific Company, Pittsburgh, Pa.). For closing the test tubes, neoprene rubber stoppers were selected whose narrow ends were smooth, flat, and circular. They were washed with chloroform, water, acetone, and water, and allowed to dry. After use, they were washed with water, acetone, and water, and dried.

All volumetric flasks and pipets used had been retested for accuracy. Lifetime Red volumetric flasks were employed in the preparation of the riboflavin standard solutions. All pipets were washed with water in an automatic pipet washer (*24*), then with acetone, and dried by drawing a current of air through them. The pipet washer was improved by M. S. Morgan of this department by substituting a Nichrome wire-gauze platform for the glass wool or beads recommended.

The heating bath consisted of glycerol which was electrically stirred and heated, and thermostatically controlled to 100° ±1° C. A wooden rack, bored to accept the test tubes but not their rims, was placed over the bath so that the surface of 3-ml. liquid samples in the test tubes would be just below the surface of the hot glycerol. Except where otherwise noted, experiments in the dark at room temperature were performed by preserving solutions in stoppered test tubes or flasks in closed Dewar flasks (1.9-liter) in a constant temperature room at 70° F. for the prescribed period of time.

REAGENTS

All reagent solutions were preserved in glass-stoppered bottles.

Sodium Fluorescein Reference Solution (0.02 microgram per ml.). Sodium fluorescein (0.1000 gram) was dissolved in cold distilled water and made up to 1 liter, 1 ml. of this solution was diluted to 100 ml. with distilled water, and then 1 ml. of the latter solution was diluted to 50 ml. These solutions were preserved in the dark at room temperature. A fresh reference solution (0.02 microgram per ml.) could be prepared each day, but it was found more convenient to adopt the following procedure. A cuvette was almost filled with some of this solution and tightly closed with a neoprene rubber stopper, and the stopper was cut off flush with the glass mouth. During 3 days at room temperature, with occasional shaking, the fluorescence diminished to about 72% of its original intensity, either through interaction with or adsorption by the neoprene rubber. This standard could then be used many times, with no noticeable change in fluorescence intensity. Immediately before each determination, the fluorometer was adjusted to give a reading of 50 scale divisions on the meter, with this reference solution in the cuvette well. The instrument setting was rechecked immediately after each determination.

Solution A (aqueous acetic acid, 5%). Glacial acetic acid (reagent, 47.5 ml.) was diluted to 1 liter with distilled water.

Solution B (boric acid–acetic acid solution, pH 2.3). Boric acid (reagent, 45 grams) was placed in a 1-liter volumetric flask, made up to 1 liter with 5% acetic acid, stirred magnetically until dissolved (about 1 hour), and again made up to 1 liter with 5% acetic acid.

1 *M* Sodium Acetate Solution. Anhydrous sodium acetate (reagent, 82 grams) was made up to 1 liter with distilled water.

Sodium Acetate–Acetic Acid Buffer (*3*) (pH 5.6). Glacial acetic acid (5.7 ml.) was diluted to 100 ml. with distilled water and to the solution were added 900 ml. of 1 *M* sodium acetate solution.

Diluent Solution (pH 3.9). Glacial acetic acid (40 ml.) was added to a mixture of 20 ml. of solution B plus 160 ml. of 1 *M* sodium acetate solution.

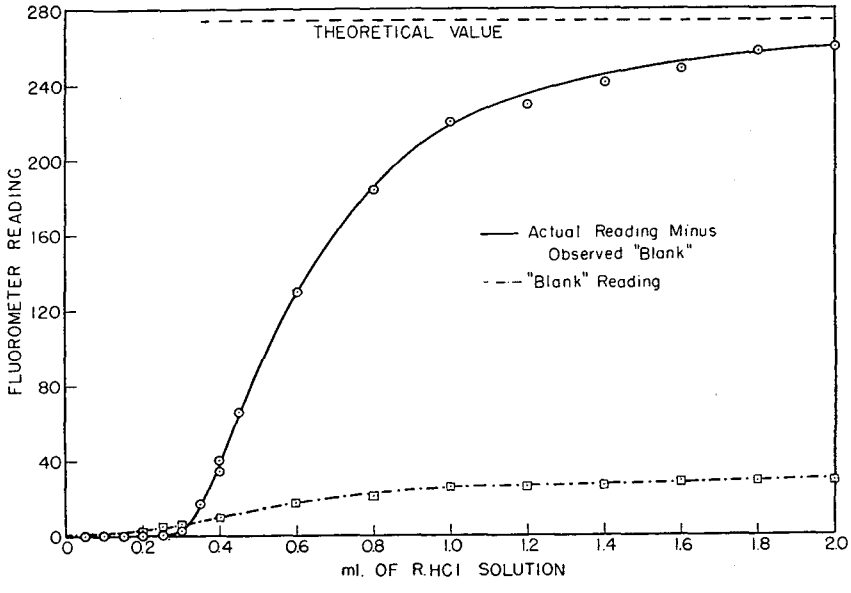


Figure 4. Effect of R.HCl on Formation of Riboflavin from 1 Microgram of Alloxan Monohydrate at Room Temperature

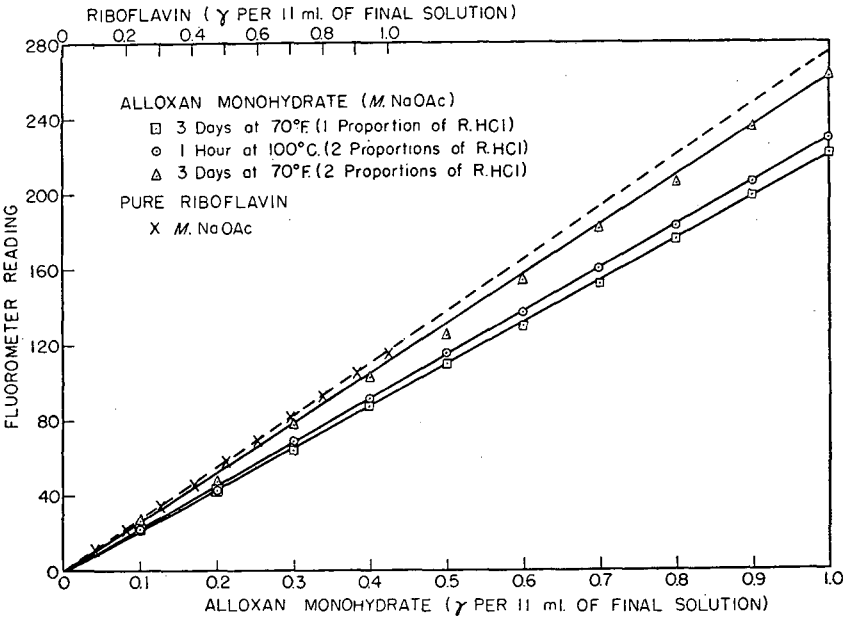


Figure 5. Calibration of Fluorometer in Terms of Alloxan Monohydrate and Riboflavin (in Solution B)

Riboflavin Solution (1.0 microgram per ml.). Riboflavin (0.0250 gram) was made up to 1 liter with solution A (or solution B) and stirred magnetically in the dark until dissolved. One milliliter of this solution was diluted to 25 ml. (red flask) with the same solvent. Riboflavin solutions were prepared fresh each day and preserved in red flasks, in the dark.

Alloxan Monohydrate Solution (1.0 microgram per ml.). As a standard, a sample of alloxan monohydrate was chosen which was colorless, and readily and completely soluble in 5 volumes of cold, distilled water. This material, which contained 3.2% of excess water, was dried to constant weight at room temperature and then preserved at room temperature in a vacuum desiccator over phosphorus pentoxide and soda lime. It remained unchanged in properties after being kept for over a year under these conditions. Alloxan monohydrate (0.1000 gram) was made up to 10 ml. with solution A (or solution B). One milliliter of this solution was diluted to 100 ml. with the same solvent; 1.0 ml. of the latter solution was diluted to 100 ml. with the same solvent. A fresh solution was prepared each day, except where otherwise noted.

R Solution (2000 micrograms per ml.). By microanalysis, R had the following elementary composition: Calculated for $C_{13}H_{22}N_2O_4$: C, 57.75; H, 8.2; N, 10.37. Found: C, 57.83; H, 7.9; N, 10.53. The base R (0.05 gram) was made up to 25 ml. with glacial acetic acid. It was preserved in a red flask in the dark. A fresh solution was prepared each day.

R.HCl Solution. Microanalysis of the hydrochloride showed the following elementary composition: Calculated for $C_{13}H_{22}N_2O_4.HCl$: N, 9.14; Cl, 11.56. Found: N, 8.69; Cl, 11.42. The hydrochloride of R (0.03 gram) was made up to 25 ml. with glacial acetic acid and shaken or magnetically stirred for 15 minutes, and the suspension was filtered through a fluted filter paper into a glass-stoppered red flask. A fresh solution was prepared each day, except where otherwise noted.

EXPERIMENTAL

Calibration of Fluorometer in Terms of Riboflavin. Into a set of nine numbered, red test tubes was pipetted 0.0 to 0.8 ml. (in increments of 0.1 ml.) of riboflavin (1.0 microgram per ml.) in solution A (or B). The volume in each tube was brought to 1.0 ml. by pipetting in 1.0 to 0.2 ml. (decrements of 0.1 ml.) of solution A (or B). To each tube were added 2.0 ml. of glacial acetic acid, followed by 8.0 ml. of the buffer solution (or of 1 *M* sodium

acetate solution). Each tube was immediately closed with a clean, dry, neoprene stopper, inverted six times, and protected from exposure to ultraviolet light. The degree of fluorescence of each sample was now determined with the fluorometer, and the fluorescence plotted against the concentration of riboflavin, giving a straight line. For the experiments employing solution B and 1 *M* sodium acetate solution, the fluorescence was also determined for 0.9 and 1.0 microgram of riboflavin; these solutions were diluted with an equal volume of diluent solution before measuring the fluorescence. A portion (5.0 ml.) of the contents (after addition of the 1 *M* sodium acetate solution) was pipetted into a cuvette containing 5.0 ml. of diluent solution. The cuvette was stoppered and inverted 6 times, and the degree of fluorescence was measured. The reading was multiplied by 2, and the reading for the "blank" subtracted. A second determination made in this way served to check the pipetting. For a given concentration of riboflavin, the degree of fluorescence was practically identical, no matter whether boric acid was present or absent, or whether buffer or 1 *M* sodium acetate solution was used. For this reason, the points for the solution B plus 1 *M* sodium acetate combination only are shown in Figure 5. The final solution had pH 3.90 (Beckman pH meter, Model H2), regardless of which of the above four combinations was employed. This is in the pH range recommended by Scott *et al.* (*20*) for fluorometric determination of riboflavin.

Solubility of Riboflavin in Solution B. Riboflavin (0.2250 gram) was made up to 1 liter with solution B, and the suspension was stirred magnetically in the dark for 15 minutes, and kept overnight in the dark at room temperature (26° C.). The suspension was stirred in the dark for the same length of time every day for a week and then a portion was filtered in the dark through a fluted filter into a red flask. The filtrate (1.0 ml.) was diluted to 100 ml. with solution B. To 1.0 ml. of this solution in a red test tube were added 2.0 ml. of glacial acetic acid and 8.0 ml. of *M* sodium acetate solution, the tube was stoppered, and the mixture was shaken. A portion of this solution (5.0 ml.) was mixed with 10.0 ml. of diluent solution and the fluorescence was observed. The reading corresponded to a concentration of 178.5 micrograms per ml. of original solution. This is more than 3 times the solubility in distilled water (*6*).

Effect of Heating Riboflavin Solution in Presence and Absence of Light. The first experiment described above was repeated, in ordinary borosilicate glass test tubes, except that, before the buffer solution was added, each tube was swirled, stoppered, and heated, under laboratory lighting plus diffuse daylight, at 100° for 3 hours. Each tube was then cooled in ice, 8.0 ml. of the buffer solution were added, and the fluorescence was measured. A control set of tubes was kept in the dark at room temperature for the same period of time. Heating in the light caused extensive disappearance of the riboflavin fluorescence (*4*, *20*).

The experiment was now repeated, except that before the solutions were pipetted into the test tubes, the latter were wrapped with tin foil to exclude light completely. Heating for 3 hours at 100° then caused no appreciable change in the fluorescence, as compared with unheated controls. When Lifetime Red Pyrex test tubes (not wrapped with tin foil) were employed, there was similarly no loss in fluorescence.

Effect of R.HCl on Riboflavin Fluorescence. Two sets of test tubes encased in tin foil were prepared as described under "Calibration," except that into each tube in one set were pipetted 1.0 ml. of glacial acetic acid and 1.0 ml. of R.HCl solution (instead of 2.0 ml. of glacial acetic acid). All the tubes were heated at 100° for 3 hours, cooled in ice, and treated as usual. After subtraction of the blank contributed by the heated R.HCl, there was no difference in fluorescence between those tubes which contained R.HCl and those which did not. The result was the same when red test tubes were employed.

Effect of Boric Acid on Riboflavin Fluorescence after Heating. Two sets of red test tubes were prepared as described under "Calibration"; in one set, solution B was used instead of solution A for preparation of the initial riboflavin solution. All the tubes were heated at 100° for 3 hours and cooled in ice, and the contents of each were diluted with 8.0 ml. of the buffer solution. There was no difference in fluorescence between tubes that contained boric acid and those that did not.

Repetition of Experiment of Banerjee *et al.* (*2*), using R.HCl. Into a set of 10 red test tubes was pipetted 0.1 to 1.0 ml. (in increments of 0.1 ml.) of fresh alloxan monohydrate solution (1.0 microgram per ml., in solution A). The volume in each tube was brought to 1.0 ml. by pipetting in 0.9 to 0.0 ml. (in decrements of 0.1 ml.) of solution A. To each tube were added 1.0 ml. of glacial acetic acid and 1.0 ml. of fresh R.HCl solution, and the tube was stoppered, swirled, and heated at 100° for 3 hours. The tubes were then cooled in ice, the contents were diluted with 8.0 ml. of buffer solution, stoppered, and inverted 6 times, and the degree of fluorescence was measured (see Figure 1). For each tube containing alloxan monohydrate, a corresponding blank was prepared (containing 1.0 ml. of solution A plus the other ingredients) and heated simultaneously. Because Banerjee *et al.* (*2*) failed to specify their reference solution, its concentration, and the instrument sensitivity they employed, the authors were unable to duplicate their instrument conditions for measurement of the fluorescence. By coincidence, the respective fluorometric readings observed for 11 ml. of final solution agreed closely with the readings they recorded for 10 ml. of solution.

Effect of Boric Acid on Yield of Riboflavin from Alloxan Monohydrate at 100° C. The preceding experiment was repeated, using solution B instead of solution A and 1 *M* sodium acetate instead of buffer (see Figure 1). In the case of tubes originally containing 0.3 to 0.8 microgram of alloxan monohydrate, 5.0 ml. of the contents (after addition of the acetate solution) were mixed with 5.0 ml. of diluent solution as previously described. For the tubes originally containing 0.9 and 1.0 microgram of alloxan monohydrate, 5.0 ml. of the contents were pipetted into 10.0 ml. of the diluent solution.

Rate of Formation of Riboflavin from Alloxan Monohydrate at 100 °C. Into each of a series of red test tubes were pipetted 1.0 ml. of fresh alloxan monohydrate solution (1.0 microgram per ml. in solution B), 1.0 ml. of glacial acetic acid, and 1.0 ml. of fresh R.HCl solution. The tubes were swirled, stoppered, and heated at 100° for various lengths of time. Allowance was made for the fact that a period of 2 minutes was required for the temperature of the contents of a tube to reach that of the bath. The tubes were then cooled in ice, the contents were treated as usual, and the degree of fluorescence was measured (see Figure 2). Each point was determined at least in duplicate. For each tube containing alloxan monohydrate, a corresponding blank was prepared (containing 1.0 ml. of solution B plus the other ingredients) and heated simultaneously. In the case of tubes (containing alloxan monohydrate) which were heated for 10 to 35 minutes, the contents (after addition of the 1 *M* sodium acetate solution) were diluted with an equal volume of diluent solution. For tubes heated longer than 35 minutes, 5.0 ml. of the contents were pipetted into 10.0 ml. of the diluent solution. From Figure 2 it is seen that, under the conditions specified, maximum formation of riboflavin occurred after 60 minutes at 100°. The quantity formed was, however, less than the theoretical.

Rate of Formation of Riboflavin from Alloxan Monohydrate at Room Temperature. The experiment was performed as in the previous one except that, instead of being heated, the stoppered tubes were kept at 70° F. in the dark for various periods of time The contents of each tube were then treated as usual, and the degree of fluorescence was determined (see Figure 2). The contents of tubes (containing alloxan monohydrate) kept for 11 to 31 hours were diluted (after addition of the 1 *M* sodium acetate solution) with an equal volume of the diluent solution, as described above; those kept for longer than 31 hours were diluted with two volumes of the diluent solution. From Figure 2 it is seen that formation of riboflavin is essentially complete after 3 days at room temperature, and that the yield of riboflavin is higher than when the condensation is conducted at 100° during 1 hour.

Effect of Pretreating Alloxan Monohydrate Solution (ca. 67% acetic acid). AT 100° C. The following procedure was adopted in order to preserve constant the depth of immersion of the heated mixture in the glycerol bath.

Into each of a series of red test tubes were pipetted 1.0 ml. of fresh alloxan monohydrate solution (1.0 microgram per ml. in solution B) and 2.0 ml. of glacial acetic acid. For each tube a corresponding blank containing 1.0 ml. of solution B plus 2.0 ml. of glacial acetic acid was prepared. The tubes were swirled, stoppered, heated at 100° for 0 to 60 minutes (in 5-minute increments, with a duplicate for each time interval), and then cooled in ice. A portion (1.5 ml.) of each of the preheated solutions was now pipetted into a red test tube, 0.5 ml. of solution B followed by 1.0 ml. of fresh R.HCl solution was added, and the tube was stoppered, swirled, and heated at 100° for 1 hour. It was then cooled in ice, the contents were diluted with 8.0 ml. of 1 *M* sodium acetate solution followed by an equal volume of diluent solution, and the fluorescence was measured. No significant change was caused by preheating the alloxan monohydrate solution for periods of up to 1 hour.

AT 70° F. The experiment was repeated except that instead of heating the initial mixtures, they were preserved at 70° F. for 3 days. Solution B and fresh R.HCl were then added as before, and the mixtures were again kept in the dark at 70° F. for 3 days. No significant change in fluorescence resulted.

Effect of Heat on Riboflavin Formed. Into each of a series of red test tubes were pipetted 1.0 ml. of fresh alloxan monohydrate solution (1.0 microgram per ml. in solution B), 1.0 ml. of glacial acetic acid, and 1.0 ml. of fresh R.HCl solution. For each tube, a corresponding blank containing no alloxan monohydrate was prepared. The tubes were swirled, stoppered, and kept at 70° F. in the dark for 3 days. Half of the tubes (and blanks) were now heated at 100° C. for 1 hour and then cooled in ice. The fluorescence of each sample, treated as usual and suitably diluted with diluent solution, was determined. The observed fluorescences for the heated reaction solutions and the heated blanks were higher than for the unheated ones, but the differences (fluorescence of reaction solution minus fluorescence of blank) were identical, indicating that the riboflavin formed was not decomposed on heating at 100° for 1 hour in a red tube. This confirms the results obtained with pure riboflavin.

Effect of Aging of Alloxan Monohydrate Stock Solution. Solutions of portions of the same sample of alloxan monohydrate (1.0 microgram per ml. in solution B) were prepared at intervals of several days and stored in the dark at room temperature. Samples of each solution were then treated as follows on the same day.

Into each of a series of red test tubes were pipetted 1.0 ml. of the respective alloxan monohydrate solution, 1.0 ml. of glacial acetic acid, and 1.0 ml. of fresh R.HCl solution. For each tube, a corresponding blank tube (containing no alloxan monohydrate) was prepared. The tubes were swirled, stoppered, and kept at 70° F. in the dark for 3 days. The contents of each tube were diluted first with 8.0 ml. of *M* sodium acetate solution and then with an equal volume of diluent solution as previously described, and the fluorescence was measured. The age of the alloxan monohydrate solution (at the time of addition of the R.HCl solution) plotted against the fluorescence developed is shown in Figure 3.

Effect of Aging of R.HCl Solution. Solutions of R.HCl in glacial acetic acid were prepared, as previously described, at intervals of several days and each was stored in a glass-stoppered red flask in the dark at room temperature. Samples of each solution were then treated as follows on the same day. Into each of a series of red test tubes were pipetted 1.0 ml. of fresh alloxan monohydrate solution (1.0 microgram per ml. in solution B), 1.0 ml. of glacial acetic acid, and 1.0 ml. of the respective R.HCl solution. For each tube, a corresponding blank tube, containing (in addition to the other liquids) 1.0 ml. of the respective R.HCl solution but no alloxan monohydrate, was prepared. The tubes were then treated as in the previous experiment. The age of the R.HCl solution (at the time of addition of the alloxan monohydrate solution) plotted against the fluorescence developed is shown in Figure 3, from which it is seen that the R.HCl solution decomposes at room temperature much more rapidly than does the alloxan monohydrate stock solution.

Effect of Various Proportions of R.HCl Solution. Into a set of 11 red test tubes were pipetted 0.0 to 2.0 ml. (in increments of 0.2 ml.) of fresh R.HCl solution and the volume in each tube was brought to 2.0 ml. by pipetting in 2.0 to 0.0 ml. (in decrements of 0.2 ml.) of glacial acetic acid. To each tube was added 1.0 ml. of fresh alloxan monohydrate solution (1.0 microgram per ml. in solution B), and the tube was stoppered, swirled, and kept at 70° F. in the dark for 3 days. For each tube, a corresponding blank tube (containing 1.0 ml. of solution B instead of 1.0 ml. of the alloxan monohydrate solution) was prepared. The contents of each tube were now diluted with 8.0 ml. of 1 *M* sodium acetate solution, and the fluorescence was measured as previously described. The contents of tubes originally containing 0.6 ml. or more of R.HCl solution were diluted with 2 volumes of diluent solution.

The results are given in Figure 4, from which it is seen that the excess of R.HCl present is not sufficient to drive the reaction to completion in all cases. The experiment was repeated in the range of 0.00 to 0.45 ml. of R.HCl solution, in increments of 0.05 ml. In Figure 4 is also shown the increase (with R.HCl concentration) in fluorescence of the various blanks at room temperature during 3 days.

Calibration of Fluorometer in Terms of Alloxan Monohydrate. Into a set of 11 red test tubes was pipetted 0.0 to 1.0 ml. (in increments of 0.1 ml.) of fresh alloxan monohydrate solution (1.0 microgram per ml. in solution B). The volume in each tube was brought to 1.0 ml. by pipetting in 1.0 to 0.0 ml. (in decrements of 0.1 ml.) of solution B. To each tube were added 1.0 ml. of glacial acetic acid and 1.0 ml. of fresh R.HCl solution, and the tube was stoppered, swirled, and kept for 3 days in the dark at 70° F. The contents of each tube were now diluted with 8.0 ml. of 1 *M* sodium acetate solution, and the fluorescence was measured as previously described. The contents of tubes originally containing 0.5 to 0.8 microgram of alloxan monohydrate were diluted with an equal volume of diluent solution; those originally containing 0.9 and 1.0 microgram of alloxan monohydrate were diluted with two volumes of diluent solution. The results are given in Figure 5. All final solutions had a pH of 3.9. The experiment was repeated, using 2.0 ml. of fresh R.HCl solution instead of 1 ml. of glacial acetic acid plus 1 ml. of R.HCl solution. The latter experiment was now repeated, except that the solutions were heated at 100° C. for 1 hour, instead of being kept at 70° F. for 72 hours (see Figure 5).

PURITY OF ALLOXAN MONOHYDRATE SAMPLES

In the course of other work, the authors have used commercial samples of alloxan monohydrate supplied by a variety of companies. Visual inspection suggested considerable variation in the purity of these samples, not only from company to company, but from the same supplier at different times. Thus, the color might vary from colorless to a uniform pink, or the sample might be mainly colorless with a small or large distribution of pink to purple particles throughout. This suggested that the alloxan monohydrate had been prepared by oxidation of alloxantin, and that this oxidation had, in many cases, been incomplete; unoxidized alloxantin had then combined with atmospheric ammonia to give the purple dye, murexide (*18*, *25*).

Table I. Purity of Commercial Specimens of Alloxan Monohydrate

Company	Sample	Color of Sample	Solubility of 10 Grams of Sample in 50 Ml. of Water	Murexide Test	% Purity (±1%, by Fluorometry)
1	D[a]	Colorless	Complete	Zero	99
	C	Extremely pale pink	Complete	Faint	98
	X	Pale pink	Almost complete	Slight	96
	Y	Very pink	Almost complete	Slight	99
	A	Very pale pink	Almost complete	Fairly strong	97
2	B	Pink	Trace of insoluble	Very strong	88
3	R	Colorless	Almost complete	Slight	96

[a] Standard sample employed in all previous experiments described herein

Again, certain samples dissolved readily and completely in 5 volumes of cold water, whereas others contained a proportion of much less soluble, colorless crystals; alloxantin is much less soluble than is alloxan in water (*25*). The highest proportion found in any sample by this procedure was 8%. This would probably not affect biological experiments employing an aqueous solution, for alloxantin dissolved or suspended in water is readily oxidized to alloxan by oxygen in the atmosphere or in water not deaerated.

In order to evaluate these qualitative observations, the method described above was applied to determination of the purity of commercial samples of alloxan monohydrate obtained from several different companies. It was found that presence of a pink color did not necessarily mean that the sample was very impure. This was presumably because, owing to its vivid and intense color, the merest trace of murexide may be detected visually; separate tests with pure murexide showed that 1 microgram per ml. is readily seen by the naked eye.

Determination of Purity of Commercial Specimens of Alloxan Monohydrate. For the qualitative murexide test shown in Table I, approximately 100 mg. of the sample were placed in a small test tube, 5 drops of concentrated ammonia were added, and the degree of murexide color formation was observed. In the rough solubility tests, 10 grams of the alloxan monohydrate sample were placed in a 125-ml. Erlenmeyer flask, 50 ml. of ordinary distilled water were added, and the mixture was shaken for 5 minutes at room temperature. Any undissolved material was removed by filtering through a fluted filter. The filtrate was used for macrodetermination of the yield of alloxantin dihydrate on reduction, by addition of a solution of 11 grams of L-ascorbic acid in 55 ml. of water (*1*). After standing overnight at room temperature, the suspension was filtered, and the crystals were dried and weighed. The yield ranged from 86 to 96% of the theoretical, depending on the purity of the alloxan monohydrate used. [Recrystallization

of 25 grams of crude alloxantin dihydrate, thus prepared, from 400 ml. of (boiled) boiling water gave 22 grams of alloxantin dihydrate.]

For the fluorometric determinations, solutions of the various samples of commercial alloxan monohydrate (1.0 microgram per ml. in solution B) were freshly prepared. A series of red test tubes was then made up to contain 1.0 ml. of the respective alloxan monohydrate solution plus 2.0 ml. of fresh R.HCl solution, at least three such tubes being prepared for each alloxan monohydrate sample. After preservation at 70° F. in the dark for 3 days, the fluorescence was determined as previously described. Two volumes of diluent solution were necessary in every case. The results are given in Table I.

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Determination of Traces of Beryllium in Biological Material

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A procedure is described for the isolation and determination of traces of beryllium in biological material. After destruction of organic material, the excess of calcium is precipitated as sulfate and beryllium is separated from the solution by precipitation with ammonia together with iron phosphate which is added as a collector. Iron and other interfering cations are removed by electrolysis with a mercury cathode, and beryllium phosphate is then separated from the solution by ammonia precipitation using aluminum as a collector. Beryllium is estimated quantitatively by the fluorometric method with morin. The smallest amount of beryllium that can be detected reliably by this method is 0.05 microgram. Because beryllium can be isolated from large samples, it can be determined quantitatively in concentrations of less than 1 part in 10^{10} parts of urine.

TOXICOLOGICAL studies of beryllium have necessitated the development of analytical methods for its determination in biological material (*1–4, 9*). Of these procedures, only the spectrographic ones (*2, 3, 9*) possess the sensitivity necessary to detect beryllium in the small concentrations present in the urine of exposed persons. Because the expensive equipment required for spectrographic determination is not available in most laboratories, the development of a sensitive chemical procedure appeared desirable. Such a method, in order to be useful for estimating the degree of exposure of workers to beryllium compounds, should, according to Cholak and Hubbard (*3*), permit the determination of beryllium in concentrations of 0.2 microgram per liter of urine. Of all reactions devised for the estimation of beryllium, only the fluorometric one with morin is sufficiently sensitive to allow quantitative estimation of such small amounts of beryllium. This method, first described by Zermatten (*11*), was carefully investigated by Sandell and adapted to the determination of beryllium in ores (*6–8*). Its use for biological material, however, was discouraged by subsequent workers (*1, 4*) because of its lack of specificity.

In order to apply the morin reaction to urine and other biological materials, complete separation of beryllium from interfering substances proved necessary. In the following report a procedure is described which permits concentration of traces of beryllium from large quantities of biological material, its separation from interfering compounds, and its fluorometric determination by the morin method.

REAGENTS

All reagents were of C.P. grade: nitric acid, concentrated; hydrochloric acid, 3 *N;* sulfuric acid, concentrated, 3 *N* and 1 *N;* hydrofluoric acid; ammonia, distilled, concentrated, and 3 *N;* sodium hydroxide, 1 *N;* a saturated solution of ammonium sulfate; potassium sulfate, 3%; bromocresol green, 0.04%; potassium ferricyanide, 1%; potassium ferrocyanide, 5%; ethyl alcohol, 95 and 60%.

Iron Phosphate Reagent (1% iron). (*a*) A 2 molar solution of phosphoric acid. (*b*) 43.2 grams of $FeNH_4(SO_4)_2.12H_2O$ and 50

ml. of 10 N sulfuric acid, diluted with water to 250 ml. Equal parts of (*a*) and (*b*) are mixed. Only a week's supply of this reagent should be mixed, because iron phosphate precipitates after this period.

Acetate Buffer, 230 grams of ammonium acetate and 270 ml. of glacial acetic acid, diluted with water to 1000 ml.

Aluminum Sulfate (1% aluminum), 12.3 grams of $Al_2(SO_4)_3 \cdot 18H_2O$ and 10 ml. of 1 N sulfuric acid, diluted to 100 ml. and filtered.

Aluminum Sulfate (0.2% aluminum), made by diluting the 1% aluminum solution with water.

Aluminate Solution. To a 50-ml. centrifuge tube are added 8 ml. of 1% aluminum solution, 5 ml. of 2 M phosphoric acid, and water to about 30 ml. After addition of 5 drops of bromocresol green, the solution is neutralized approximately with ammonia and 5 ml. of acetate buffer are added. It is heated in boiling water bath for 10 minutes, and centrifuged, and the precipitate is washed twice with 3% potassium sulfate. The precipitate is dissolved in 40 ml. of 1 N sodium hydroxide, transferred to a 100-ml. volumetric flask, diluted to volume with water, filtered, and kept in a glass-stoppered bottle.

Stannite Solution. A 1% solution of stannous chloride dihydrate in 0.5 N sodium hydroxide is filtered and kept in a glass-stoppered bottle.

Morin Solution. A stock solution is made by shaking 500 mg. of finely ground morin (Eastman, technical) with 100 ml. of 95% alcohol overnight, and filtering the saturated solution. This solution keeps at least 3 months in the refrigerator. The working reagent is made by diluting 5 ml. of the stock solution with water to 100 ml. It is kept in glass-stoppered bottles.

Beryllium Standard Solution. A concentrated standard containing 10 micrograms of beryllium per ml. is made by dissolving 196.6 mg. of beryllium sulfate tetrahydrate in water, adding 10 ml. of 3 N hydrochloric acid, and diluting to 1 liter. The dilute working standard containing 0.2 microgram per ml. is made by diluting 2 ml. of this solution to 100 ml. with water. If kept in borosilicate glass bottles, these solutions will not change their titer.

PROCEDURE FOR DETERMINATION OF BERYLLIUM IN URINE

For determination of minute amounts of beryllium such as may occur in the urine of exposed persons, it is best to ash a specimen collected during a 24-hour period, regardless of its volume. Ordinary dry-ashing procedures cannot be used, as shown by Cholak and Hubbard (*2*), presumably because of the volatility of beryllium chloride. Losses are avoided, however, if hydrochloric acid is first expelled by evaporating with nitric acid.

The urine is transferred from its original container into one or more large Erlenmeyer flasks and the container is rinsed with a total volume of 100 ml. of concentrated nitric acid. The combined urine and nitric acid rinsings are concentrated on a hot plate. If, on boiling, the mixture does not turn a light yellow and boiling becomes uneven, more nitric acid must be added. When the volume has been reduced sufficiently, the solution is transferred to a 350-ml. Vycor evaporating dish and taken to dryness on a steam bath. The contents of the dish are then moistened with 10 ml. of concentrated nitric acid, and the dish is placed into a radiator and heated over a low flame. (Iron containers 10 cm. in diameter and 4 cm. in height, as used for packing 100-foot rolls of 35-mm. photographic film, are ideal for this purpose.) When the mixture becomes dry, the flame is gradually increased until charring occurs and no vapors are given off. The dish is then heated in a muffle furnace to 500° C. until the ash is pure white.

The ash is dissolved in a minimal amount of 3 N hydrochloric acid, usually less than 100 ml., and heated until the small amount of silica coagulates. It is then filtered through a small ashless paper and washed once with water and twice with dilute (1 N) sulfuric acid. The paper is ignited in a platinum crucible and to the residue are added a few drops of concentrated sulfuric acid and about 1 ml. of hydrofluoric acid. This is heated until dense fumes of sulfuric acid appear, water is added, and the clear solution is combined with the main filtrate. The beaker containing this filtrate is then covered with a watch glass, heated, and kept boiling for 20 minutes in order to convert any meta- and pyrophosphates to orthophosphates. A few glass beads may be added to prevent bumping. If, on cooling, crystals are formed, they are brought back in solution by the addition of water.

Then 10 ml. of saturated ammonium sulfate solution are added for every 50 ml. of the solution and calcium sulfate is precipitated by adding 1.3 volumes of 95% alcohol. The solution is allowed to stand for about 30 minutes and filtered through a Büchner funnel, and the precipitate is washed two or three times with small quantities of 60% alcohol. The filtrate and washings are evaporated in a beaker on a water bath either until most of the alcohol has been eliminated or, more conveniently, overnight to dryness. If dry, the salts are dissolved in water and acidified with a small amount of hydrochloric acid.

To this solution, from which most of the calcium has been removed, 2 ml. of iron phosphate reagent are added, followed by enough concentrated ammonia to cause fading of the yellow color of ferric chloride. Before precipitation of iron phosphate begins, 10 drops of bromocresol green are added and the solution is neutralized under constant stirring by slowly adding dilute (3 N) ammonia until the indicator changes to light green. About 1 volume of acetate buffer is then added for every 5 volumes of solution, and the mixture is heated to near boiling and placed on the steam bath for 10 minutes. The solution should not be boiled or heated on the steam bath for a prolonged period, as volatilization of acetic acid may cause appreciable change in the hydrogen ion concentration. The precipitate which contains the beryllium is then collected in a conical 50-ml. centrifuge tube by repeated centrifugation and washed twice with 40 ml. of 3% potassium sulfate solution. It is important to disperse the precipitate completely in the washing solution, which is best done with the help of a thin-footed glass rod. Between washings the centrifuge tube is inverted and drained on a pad of filter paper. After the last washing the precipitate is dissolved in 1 ml. of 3 N sulfuric acid.

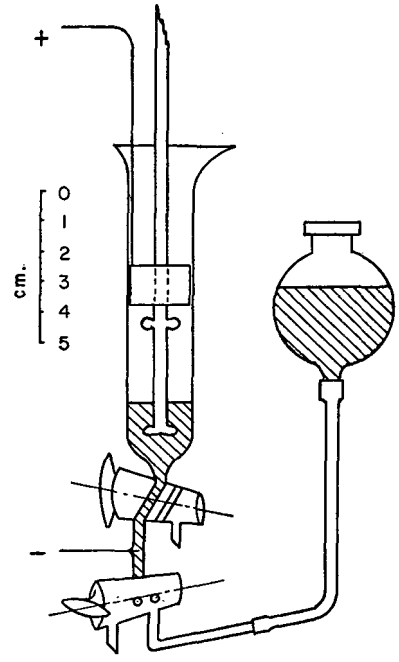

Figure 1. Cell for Electrolysis with Mercury Cathode

The electrolysis vessel shown in Figure 1 has proved particularly convenient for electrolysis of small volumes of solution. This cell, a modification of the one described by Melaven (*5*), permits separation of the electrolyte from the mercury without the use of a leveling bulb. [Recently an electrolysis cell has been described in which the mercury is separated from the solution by a somewhat similar stopcock arrangement (*10*).] In addition to the greater ease of operation, this has the advantage that contaminated mercury is never returned to the reservoir. (Used mercury may be cleaned sufficiently by aeration.)

For electrolysis enough mercury is run into the cell from the reservoir to cover the lower paddle of the motor-driven stirrer and the flow of mercury is stopped by closing the lower stopcock while the upper remains open. The sulfuric acid solution of the phosphates is then transferred to the electrolysis vessel with the help of several small washings with water. The volume at this point should be about 9 ml. The platinum anode is adjusted to the top level of the solution and electrolysis is carried out with stirring, using a current of 0.2 to 0.3 ampere (a direct current source of about 20 volts is required). When most of the iron has been re-

Table I. Recovery of Beryllium Added to 24-Hour Urine Collections and to Tissues of Individuals Not Exposed to Beryllium

Sample	Amount	Weight of Ash	Beryllium Added	Beryllium Found	Recovery
	Ml.	*Grams*	*γ*	*γ*	*%*
Urine	1300	14.9	0	0	...
	1700	16.5	0	0	...
	820	11.5	0.05	0.05	100[a]
	910	12.8	0.05	0.05	100[a]
	1500	16.5	0.2	0.16	80
	1500	13.9	0.2	0.17	85
	1650	10.9	0.4	0.34	85
	1300	16.4	0.4	0.33	83
	2000	17.2	0.6	0.47	78
	1900	13.1	0.6	0.43	72
	1900	12.0	1.0	0.8	80
	1900	14.5	1.0	0.67	67
	2200	13.2	1.2	1.06	88
	2600	20.0	1.2	0.96	80
	Grams				
Liver	53	0.4	0.4	0.34	85
	20	0.1	0.4	0.33	83
Bone	13	6.6	0.5	0.41	82
	25	14.0	0.5	0.40	80
				Av.	80.5

[a] Because of relatively large error in determination of very small intensities of fluorescence, these values are not included in average.

Table II. Recovery of Beryllium Added at Various Stages during Analysis of Beryllium-Free Urine Samples

0.5 γ Beryllium Added before:	Beryllium Recovered	
	γ	*%*
Ashing	0.42	84
$CaSO_4$ precipitation	0.43	86
Precipitation of beryllium and iron phosphate	0.43	86
Precipitation of beryllium and aluminum phosphate	0.46	92

moved (about 10 minutes) 2 ml. of 1 *N* sodium hydroxide are added to the electrolysis vessel, and any spray is rinsed from the sides of the vessel and from the stirring rod. Electrolysis is then continued until spot tests with ferrocyanide as well as with ferricyanide are negative. This usually takes another 10 minutes. At this point the spray is again washed down with a small portion of water and electrolysis is continued for another 15 minutes. The mercury is then lowered to the level of the upper stopcock by draining it through the lower stopcock. During this process the anode is lowered in order not to interrupt the flow of current. The solution is then transferred to a conical 30-ml. centrifuge tube through the open end of the upper stopcock, and the electrolysis vessel is washed with repeated small portions of water. The volume of solution and washings should be 15 ml.

To the iron-free solution are added 1 ml. of 0.2% aluminum solution and 2 drops of bromocresol green. Neutralization with dilute (3 *N*) ammonia is then carried out slowly and with vigorous stirring until the first permanent precipitate of aluminum phosphate forms. The indicator at this point should still be yellow. While stirring is continued 3 ml. of acetate buffer are added. The pH of the solution will then be exactly 4.4. The centrifuge tube is placed in a boiling water bath for 5 minutes and centrifuged. The supernatant liquid is decanted and the precipitate is suspended in 15 ml. of 3% potassium sulfate solution with the help of a footed stirring rod. The tube is then placed in a boiling water bath until the precipitate coagulates and is centrifuged while hot. The washing procedure is repeated a second time.

The precipitate of aluminum phosphate and beryllium is dissolved in 1 ml. of 1 *N* sodium hydroxide and heated briefly in a boiling water bath. It is then diluted to 10 ml. with water and centrifuged. If the procedure has been carried out correctly, there will be at this point only a minimal amount of undissolved residue which consists of silica. Four milliliters of the clear solution are pipetted into an 18-mm. test tube, followed by 1 ml. of stannite solution. At the same time the blank is prepared by adding 1 ml. of aluminate solution and 1 ml. of stannite solution to 3 ml. of water. The standard contains 1 ml. of aluminate, 1 ml. of stannite, 1 ml. of standard beryllium solution (0.2 microgram), and 2 ml. of water. These solutions are allowed to stand at room temperature for about 15 minutes, after which 1 ml. of morin solution is added to all tubes simultaneously. The authors have measured the fluorescence with a Beckman photofluorometer, setting the blank at 0 and the standard to 100 on the transmittance scale with the selector switch in the 0.1 position. Because low intensities of fluorescence are measured, it is necessary to calibrate the cells and to apply the usual corrections to the readings. If the reading is higher than 100, the determination is repeated, using a smaller aliquot of the unknown solution. To this aliquot (n ml.) are added an amount of aluminate solution equal to $\frac{4-n}{4}$ ml. and enough water to bring the volume to 4 ml. The amount of beryllium in the original sample is $\frac{R}{100} \times 0.2 \times \frac{10}{n} \times \frac{1}{0.8}$ micrograms, where R is the corrected reading and n the volume of aliquot in milliliters. The factor of $\frac{1}{0.8}$ is introduced to correct for the loss of beryllium, because the average recovery during the procedure is 80% (see Table I).

DETERMINATION OF BERYLLIUM IN OTHER BIOLOGICAL MATERIAL

The procedure outlined above is easily adapted to the determination of beryllium in all kinds of biological material, including bone.

The tissue to be analyzed is weighed and dissolved in 50% nitric acid, taken to dryness, and ashed as described for urine. If heating in the muffle to 500° for 2 hours does not provide a clean white ash, the ash is moistened with a small amount of nitric acid or 30% hydrogen peroxide and again heated to 500°. The amount of tissue which can be subjected to analysis is essentially limited by the inconvenience of ashing large quantities. Up to 50 grams of soft tissues or bone have been ashed, however, without undue difficulty. The ash is treated exactly as in the procedure described for urine, except that in the case of most tissues other than bone the preliminary separation of calcium sulfate may be omitted, because small concentrations of calcium are not carried down by the iron phosphate precipitate.

When small amounts of tissues (<2 grams) or urine (<20 ml.) are analyzed, the conventional wet-ashing procedure with sulfuric and nitric acids is convenient. The resulting solution is diluted until the concentration of sulfuric is about 10%. In case the sample contains considerable amounts of calcium, 8 ml. of 3 *N* ammonia are added for every 10 ml. of solution and calcium is precipitated by the addition of alcohol as described. If only a few milligrams of calcium are present, precipitation of calcium sulfate is unnecessary. During the wet-ashing procedure all phosphorus remains in the form of orthophosphate, and the heating period can therefore be omitted. In every case the remaining procedure is carried out exactly as described above; the amount of iron phosphate solution added is independent of the size or volume of the sample.

Ashing with sulfuric acid is not permissible if enough calcium is present to precipitate during the ashing procedure, because, as shown by Aldridge and Liddell (*1*), this precipitate may occlude beryllium. Wet ashing is not recommended for lung tissue which may contain appreciable amounts of silica, which if not eliminated would retain beryllium during the final stages of the analysis.

RECOVERY

Table I contains unselected results obtained from consecutive analyses of daily collections of urine from persons not exposed to beryllium. These data show that no beryllium was found in such urines; however, when beryllium was added, its recovery averaged 80%, most values falling within 10% of this average. It is apparent that over the range of concentrations tested the amount of beryllium lost during the procedure is a rather constant fraction of the amount of beryllium initially present. The table also contains data on analyses of liver and bone, demonstrating that recoveries from tissues are of the same magnitude.

Table II represents the average of two series of experiments in which 0.5 microgram of beryllium was added at various stages during the analyses of urine that contained no beryllium. The data indicate that 16% of the beryllium was lost when added before ashing; 14% of the loss occurred during the coprecipitations of beryllium with iron and aluminum phosphate. The losses during ashing and separation of calcium sulfate amounted to only 2%. [The authors have recently had occasion to test the proce-

dure with the help of radioactive beryllium (Be^7). The recoveries of added radioactivity agreed well with those obtained by the chemical method.]

The procedure has been designed primarily for the analysis of material derived from industrial workers. Such material may contain elements not ordinarily encountered in biological material. Industrial exposure to beryllium is frequently associated with exposure to zinc or copper. Sandell (7) has shown that zinc causes fluorescence with morin, while copper destroys the fluorescence by oxidation of the dye. The authors have tested for possible interference by adding 10 mg. of these two metals as sulfates to urine. As expected, they were quantitatively eliminated by electrolysis and did not affect the recovery of beryllium.

COMMENTS

Precipitation of traces of beryllium phosphate with iron or aluminum phosphate as collectors has proved to be successful, provided that the hydrogen ion concentration is kept close to a pH of 4.4. If the pH is lower than this value, precipitation of beryllium is less complete, while at a higher pH the precipitate becomes contaminated with traces of calcium and possibly other alkaline earth metals. The addition of a concentrated buffer solution makes it easy to achieve the exact desired acidity. The authors have not been able to improve the recovery of beryllium by variation of the concentrations of hydrogen ions, phosphate, or ammonium salts during precipitation. The phosphate precipitates are readily separated by centrifuging, provided that pyro- or metaphosphates which may originate during dry ashing are completely hydrolyzed. Incomplete hydrolysis usually is shown by a failure of the iron phosphate to go into solution in sulfuric acid, or by a colloidal behavior of the aluminum precipitate, the latter complication causing drastic losses of beryllium.

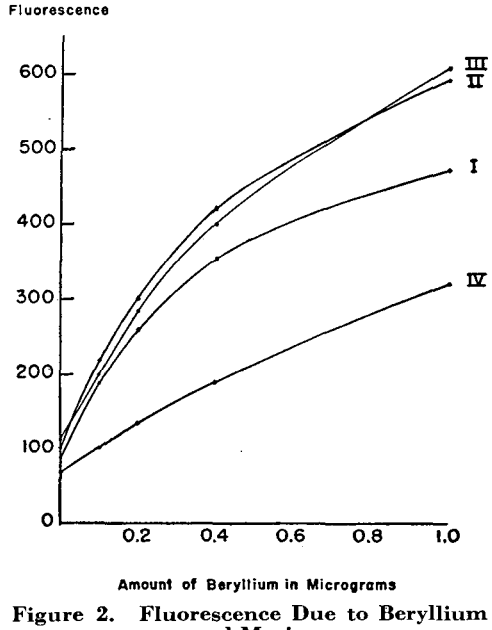

Figure 2. Fluorescence Due to Beryllium and Morin

Readings taken with Beckman photofluorometer using water as a blank solution and a permanent fluorescent standard as reference

I 0.25 ml. morin solution
II 0.5 ml. morin solution
III 1 ml. morin solution
IV 2 ml. morin solution

All tubes contained 1 ml. of aluminate, 1 ml. of stannite, and a total volume of 6 ml.

Table III. Determination of Beryllium in Pure Solution

Each tube contains 1 ml. of stannite solution, 1 ml. of aluminate solution, and 1 ml. of morin solution in addition to indicated amount of beryllium. Total volume is 6 ml.

Addition, γ Be	Fluorometric Reading	Recovery, γ Be	Addition, γ Be	Fluorometric Reading	Recovery, γ Be
0	0	0	0.1	51.5	0.103
0	0	0	0.15	74.0	0.148
0.05	25.0	0.050	0.15	75.0	0.150
0.05	26.0	0.052	0.2	100.0	0.200
0.1	49.5	0.099	0.2	100.0	0.200

The conditions for the final estimation of beryllium by means of fluorescence with morin were chosen for greatest reproducibility and stability. Figure 2 shows the fluorescence of increasing amounts of beryllium with varying concentrations of morin solution. Fluorometric readings were taken in arbitrary units, using water as a blank and a permanent fluorescent standard as reference. In the presence of small amounts of beryllium the sensitivity of the reaction increases with decreasing concentrations of dye, reaching a maximum in the presence of 0.5 ml. of morin solution (curve II). With still lower concentrations of dye the intensity of the fluorescence decreases (curve I). The addition of 1 ml. of morin solution (curve III) was adopted for the analytical procedure because in this case the relation of fluorescence to the beryllium concentration is linear up to 0.2 microgram of beryllium (see Table III), and because the degree of fluorescence is nearly independent of slight variations in the concentration of the dye. (Because the shape of fluorescence curves such as those in Figure 2 depends to some extent on the dimensions of the cuvette, a different concentration of morin might prove advantageous where a different instrument is used for measurement. Commercial morin is impure and different lots may necessitate slight alterations in the preparation of the reagent.)

It is probable that with smaller concentrations of morin, as recommended by Sandell, smaller amounts of beryllium could be detected, because the amount of fluorescence in absence of beryllium is thereby reduced. The concentration of sodium hydroxide in the final mixture was chosen so that a slight variation in the alkalinity does not affect the fluorescence appreciably, although slightly greater sensitivity could have been achieved by decreasing the concentration of alkali. Stannous chloride is added to the reaction mixture in order to stabilize the fluorescence. Under the conditions described, no appreciable fading occurs during 60 minutes.

The authors have never observed detectable traces of beryllium as contaminants of the reagents used. However, extreme precautions are indicated to avoid contamination of glassware with beryllium. Care must be taken not to introduce fluorescent material, such as may be derived from rubber or from the skin, particularly, during the final stages of the analysis. When these precautions were observed, analyses of salt mixtures known to contain no beryllium have always yielded final fluorescence readings which agreed with the blank within limits corresponding to ±0.025 microgram of beryllium. It is only this variability in fluorescence of the reagents in the absence of beryllium which sets a lower limit to the amount of beryllium that can be determined. The smallest amount which can be detected with reliability by this method is therefore at least 0.05 microgram.

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NOTES ON ANALYTICAL PROCEDURES . . .

Crossing Over

A New Technique in Displacement Analysis with The Tiselius-Claesson Interferometer

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THE Tiselius-Claesson interferometric adsorption analysis apparatus has proved to be an invaluable aid in the development of chromatographic techniques (*4*). However, the instrument has the inherent disadvantage that the range of concentrations which may be measured conveniently in any given experiment is limited. Thus, for example, the modification of the apparatus now in use in this laboratory can be used to measure fatty acid concentrations from 0 to 2.0% in alcohol solutions if both micrometer screws are used. However, it frequently is desirable to work with much more concentrated solutions, particularly in preparative experiments. The apparatus and techniques herein described make it possible to handle much wider ranges of concentration without changes in apparatus within a given experiment, thereby removing the principal limitation of the apparatus.

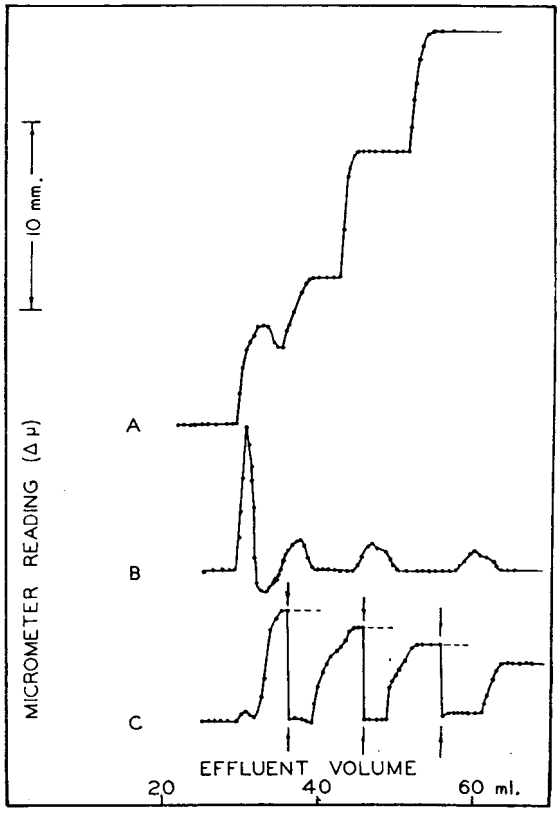

Figure 1. Displacement of Lauric, Myristic, and Palmitic Acids by Stearic Acid

Displacer 1.0% stearic acid. Solvent 95% ethyl alcohol. Filter column 5000 + 2000 + 800 + 400 + 200 + 100π cu. mm. = 26.7 ml. *A*. 20 mg. of lauric, 40 mg. of myristic, and 80 mg. of palmitic acids *B*, *C*. 30 mg. of lauric, 60 mg. of myristic and 120 mg. of palmitic acids

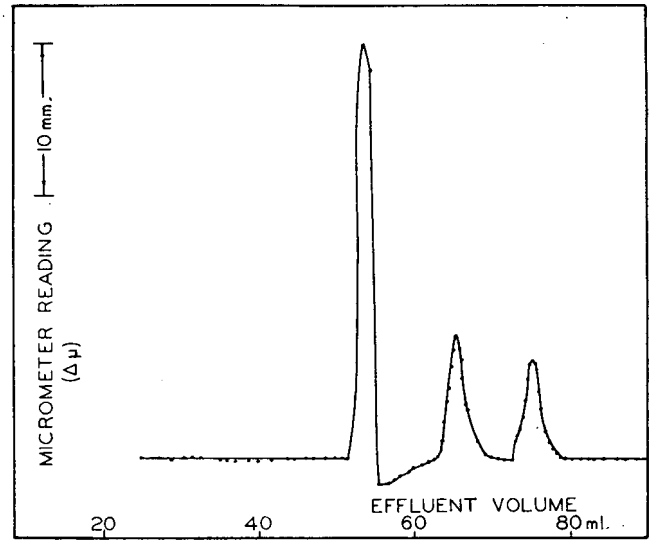

Figure 2. Displacement of Lauric and Myristic Acids by Palmitic Acid

Displacer 3.0% palmitic acid. Solvent 95% ethyl alcohol. Filter column 10,000 + 5000 + 2000 + 800 + 400 + 200 + 100π cu. mm. = 58.1 ml. Sample 250 mg. of lauric and 250 mg. of myristic acids

The Tiselius-Claesson apparatus employs an observation cuvette which has two channels through which parallel light passes. One channel carries the effluent of the chromatographic column, the other ordinarily contains a sample of the solvent used in the experiment. The concentration of the solute in the effluent is determined by the interferometric measurement of the difference in refractive index between the liquids in the two channels. The range of concentrations that can be measured is controlled by the variation in optical path provided by the rotation of the compensator glasses. Claesson has increased the range of measurement by introducing additional glass plates (*1*), but in general this device has not been entirely satisfactory. The range can be extended also by changing the liquid in the comparison tube, or by the use of shorter cuvettes.

By introducing the effluent liquid into the comparison tube, one can accomplish the same effect. If the effluent is allowed to traverse both observation channels at a time when the concentration of solute in the effluent is constant, the difference in refractive index between the contents of the two channels becomes zero, and the interference fringes return to the null position, or the base line value. If this is done repeatedly, one can follow stepwise large ranges in concentration in a displacement experiment.

As an example of this type of observation, similar displacements have been made using the different methods of observation. Curve *A* of Figure 1 represents the displacement of lauric, myristic, and palmitic acids by stearic acid, the observation being made in the customary manner. All components except lauric acid emerged in well defined steps, emerging in equilibrium concentration. A similar experiment was made using repeated crossing over, once for each component (curve *C*). When the first component emerged, the effluent was passed through the com-

parison tube (downward arrow) until the micrometer reading reached the base line value. At that time the flow through the comparison tube was stopped (upward arrow), the comparison tube was again closed, and the effluent was collected in the graduated vessel as usual. This procedure of crossing over and crossing back was repeated when each succeeding component emerged and reached its equilibrium concentration. The effect was to produce a curve, each step of which arose from the base line. Presumably, in a large chromatographic experiment, this process could be repeated indefinitely, if no single step up in concentration exceeded the range of the micrometer screws.

If at the beginning of a displacement analysis experiment the two channels are connected so that the effluent traverses both of them, the observations made then represent concentration gradients across a volume interval of effluent equal to the volume of the capillary connecting the two channels. The curve obtained then represents the rate of change of concentration, a rough differentiation of the curve obtained with the ordinary technique (curve *A*). To make the differences in concentration in the two channels conveniently measurable, a spacer can be introduced between the two channels. The spacer used in experiment *B* is a flexible capillary tube of approximately 2-ml. volume. Curve *B* shows that as each component emerged, the refractive index increment across the spacer rose sharply until the equilibrium concentration of that component had emerged. At that time the increment decreased roughly to zero. The refractive index increment decreased to a negative value on the first component, as could be predicted from the shape of curve *A*.

The latter type of manipulation, crossing over, is particularly useful in preparative experiments where the localization of the steps or components is more important than the measurement of the equilibrium concentrations, and where the use of high concentrations is desirable. In Figure 2 is shown a displacement of lauric and myristic acids by a 3.0% solution of palmitic acid. This displacer concentration is three times as high as that normally heretofore used in fatty acid separations (*2*, *3*). The three components of the displacer diagram are clearly apparent in the differential diagram, and the range of measurement has remained within the scope of the instrument. Curves obtained with the crossing over technique (Figure 1,*B*, and Figure 2) representing concentration gradients are basically similar to those obtained with the Tiselius electrophoresis apparatus. It is likely that quantitative measurements based upon the integration of these curves can also be made, thus extending the analytical use of existing instruments to higher concentration ranges.

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Amperometric Titration Cell for Use with Dropping Mercury Electrode

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AMPEROMETRIC titrations with the dropping mercury electrode suffer from the disadvantage that they must usually be performed in a solution that is free of oxygen. This condition is usually attained by passing an inert gas through the solution for a few minutes after each addition of reagent.

The necessity of repeated bubbling to remove oxygen from the solution may be obviated by maintaining oxygen-free reagents under an inert atmosphere of hydrogen or nitrogen. The solution to be titrated is freed of oxygen before the titration and is then titrated with the oxygen-free reagent; the solution is stirred between additions of reagent. The apparatus of Kolthoff and Langer (*2*) is a good example of this approach to the problem. For many reagents which are stable in air, however, this method involves an unnecessarily cumbersome type of apparatus. Because a means of stirring between increments must be provided, the gas stream used for this purpose might as well also serve to remove the oxygen introduced with the reagent.

Another approach to the problem is the designing of a cell in which the titration may be performed while a continuous flow of inert gas is passed through the cell. This continuous gas flow would provide for both oxygen removal and thorough mixing of the solution.

A primary requirement of such a cell would be an effective means of shielding the dropping mercury electrode from the flow of gas. Such shields have been described by Wise (*6*) and by Laitinen, Higuchi, and Czuha (*4*) in other analytical applications of the dropping electrode.

APPARATUS

The amperometric titration cell designed to utilize the principle of continuous gas flow for oxygen removal and solution mixing is shown in Figure 1. The flow of nitrogen (or hydrogen) gas enters through the side arm and is controlled by the 2-mm. stopcock, by means of which it may be shut off or allowed to pass through the coarse fritted-glass disk in the bottom of the cell.

The main body of the cell is about 16 cm. high (from the fritted-glass disk) and 3 cm. in diameter, and has a total capacity of about 100 ml.

The dropping electrode shield consists of two parts—an outer shield and an inner shield. The outer shield, which is suspended from the top of the cell by means of a stiff platinum wire, was made by cutting off a weighing tube, 22 mm. in diameter, about 3.75 cm. from the bottom by means of a Carborundum glass saw. By the same method, four slits, just above and parallel to the glass bottom of the tube, were cut at equal distances from each other. These slits were about 0.5 cm. long and 1.5 mm. wide. Four similar slits were made 2 cm. from the bottom of the tube but not directly above those made at the bottom. Finally, this outer shield was notched, by means of the saw, so that the platinum wire, by means of which it was suspended from the top of the cell, could be firmly attached.

The inner shield was made from a piece of glass tubing with an inside diameter about 3 mm. greater than the diameter of the dropping electrode used. The tube was cut about 2.5 cm. long and six slits, 0.5 cm. in length, were made at one end parallel to the length of the tube. Two other slits, parallel to the ends of the tube, were cut half-way between the perpendicular slits and the top of the tube. These slits, which are about 0.5 cm. long, were cut on opposite sides of the tube. This inner shield was placed inside the outer shield and when in use the dropping electrode was inserted into it to a distance about half-way between the parallel upper slits and the perpendicular slits.

Three holes were bored in a rubber stopper of suitable size to fit the top of the cell and cut off, if necessary, so that it extended only a short distance into the cell. The hole made for the insertion of the dropping electrode was bored so that the electrode fitted tightly. The stopper was left permanently attached to the electrode, which was inserted far enough into the cell so that it extended into the shield the proper distance when the stopper was in place. The other two holes were provided for the insertion of a salt bridge from a saturated calomel electrode and the tip of the buret to be used in the titration.

The end of the salt bridge was made from a glass tube, one end of which consisted of a porous fritted-glass disk and which was partially filled with an agar plug made from a 3% agar solution saturated with potassium chloride. This tube was then attached by means of a piece of rubber tubing, as described by Hume and Harris (*1*), to a saturated calomel cell made from a bottle large enough (5 to 7.5 cm., 2 to 3 inches, in diameter) to give a reference electrode which would not become polarized by the passage of the current.

[1] Present address, Standard Oil Company, Inc., Whiting, Ind.

A simple circuit for current measurements is shown in Figure 2. The applied e.m.f. was measured by a high resistance voltmeter, V. A microammeter, A, with a scale reading of 0 to 50 μa, was provided with a 2000-ohm Ayrton shunt, S, to control its sensitivity. The damping circuit of Philbrook and Grubb (*5*), utilizing two 1000-mfd. electrolytic condensers, was found effective for practical work.

OPERATION

The solution to be titrated should be placed in the cell only while the gas is passing through the cell; otherwise some solution will pass below the fritted-glass disk, spoiling the titration. If solution does get below the disk it may be effectively removed by putting suction on the side arm and rinsing the cell several times with water and finally with alcohol or acetone, after which it is sucked dry. The gas should be shut off during insertion of the electrode to prevent air bubbles from attaching themselves to the end of the capillary.

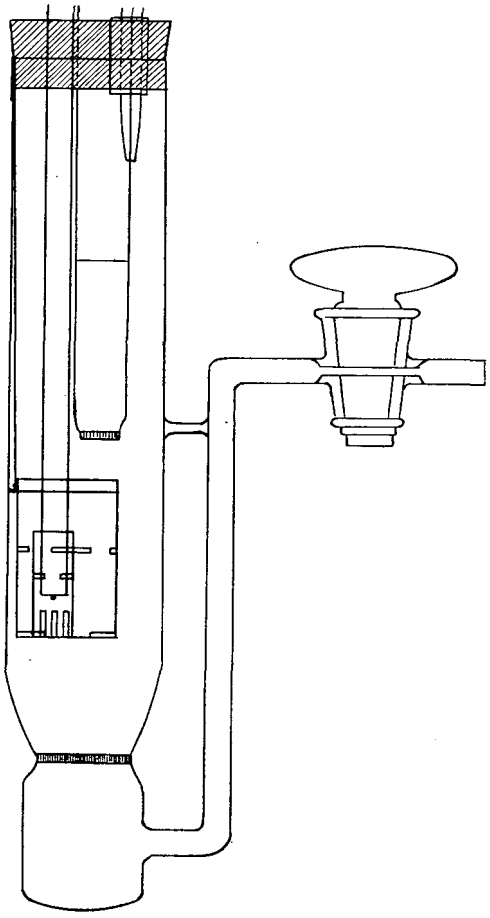

Figure 1. Amperometric Titration Cell

The cell may be permanently clamped in place, inasmuch as a most convenient way of cleaning the cell after each titration is to suck out the solution by means of a vacuum line. A trap placed in the line catches the waste solution and mercury as well as rinse water used to rinse out the cell. If necessary, a test-tube brush may be used in cleaning the cell while it is still in place. The nitrogen should be left flowing at all times during the cleaning and the glass tubing used to suck out the solutions should not be extended to the bottom of the cell until the last of the solution is being sucked out. This is necessary because the suction may reduce the gas pressure below the fritted-glass

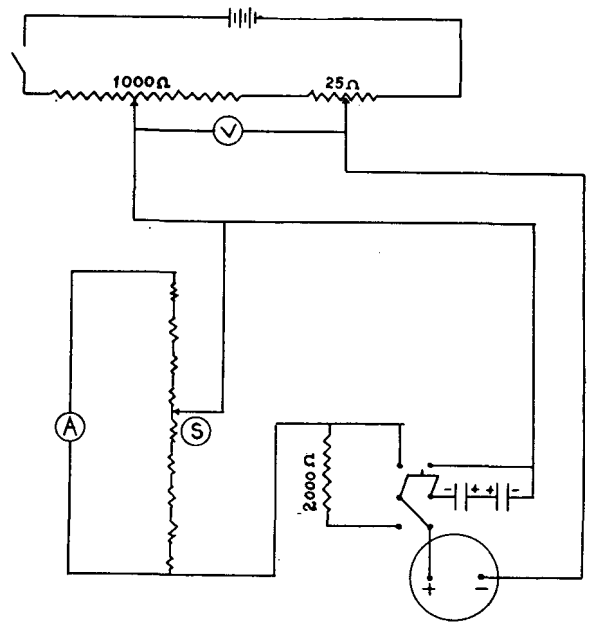

Figure 2. Diagram of Circuit

A. Microammeter
V. Voltmeter
S. Ayrton shunt

plate to a point where some solution will be allowed to pass through the plate.

A liquid pressure valve consisting of a column of water was found advantageous in controlling the gas flow at the cell rather than at the gas cylinder.

Table I. Time Required for Removal of Oxygen from 50 Ml. of 0.1 *N* Air-Saturated Potassium Chloride

(As indicated by oxygen diffusion current measurements)

Solution	Current, μa.	Time, Sec.
50 ml. of air-saturated 0.1 *N* potassium chloride	3.0	0
	0.6	30
	0.4	60
	0.4	90
10 ml. of above solution added after removal of oxygen from original 50 ml.	0.4	<5[a]

[a] From 10 ml. of air-saturated solution after completion of its addition to air-free solution.

A buret which has a flexible tip and is more adaptable to the geometry of the cell may be made by cutting off the tip of a buret about 2.5 cm. below the stopcock. The two pieces are then connected, by means of two rubber connections, to a piece of glass bent at right angles at each end. The bent ends are about 2.5 cm. long and are bent in opposite directions from each other. The center portion of the tubing may be as long as convenient, but is usually not more than 5 to 7.5 cm. long.

DISCUSSION

A coarse fritted-glass disk was found to be more satisfactory in construction of the cell than one of fine or medium porosity because of the much greater facility with which the rate of gas flow is controlled. In addition, a liquid pressure valve was found to be impractical with either of the latter porosities because of the increased liquid depth required.

Oxygen removal was effective, as shown by the data in Table I. Essentially all the oxygen was removed by passing gas through the solution for 30 seconds.

The rate of mixing was satisfactory, as indicated by the speed

with which several drops of dye were diffused throughout the cell after being placed inside the glass shield, as well as by the rate with which diffusion current readings became constant in performance of titrations.

The stability of current readings was tested by titration of sulfate ion with lead nitrate solution, according to the procedure of Kolthoff and Pan (*3*), and found to be satisfactory. Results were within the limits of accuracy specified for the method.

Current readings with gas flowing through the cell were not noticeably higher than those obtained with no gas flowing.

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Pressure Pycnometer

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DURING an investigation in this laboratory it was necessary to devise some means of determining the composition of binary liquid mixtures. Measurement of the density of the liquid mixture was chosen as the analytical tool. Because one or both of the components in the determinations were extremely volatile—e.g., CF_2Cl_2, $CHCl_2F$—ordinary pycnometric methods would not suffice.

The pycnometer used by Walden (*10*), somewhat similar to that used by Goodhue and Hazen (*4*), consisted of a glass bulb on the lower end of a graduated capillary tube. The upper end was sealed by mechanical pressure to a concentric valve of the aerosol type. The maintenance, operation, and cleaning of this model were rather difficult, and in general it was not rugged enough for the purpose.

The possibility of an all-metal construction was then investigated. The steel pycnometer used by Eilarts, Smith, and Cook (*3*) for specific volume determinations on oil-well samples was designed for use at extremely high pressures (up to 4500 pounds per square inch) and in design and operation was deemed to be too involved for adaptation to the authors' use.

The pycnometer as finally designed and used is constructed chiefly of brass, and has been used successfully at pressures up to 100 pounds per square inch.

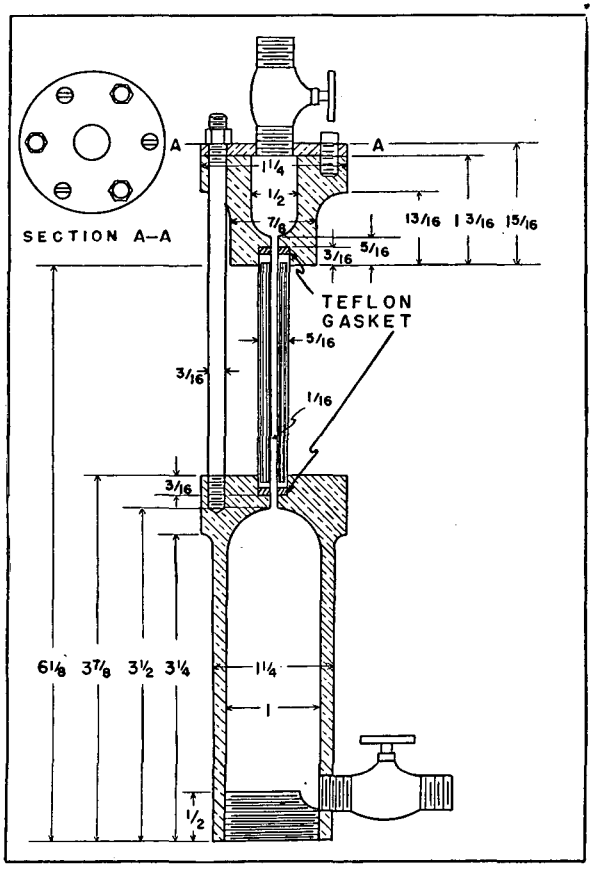

Figure 1. Diagram of Pressure Pycnometer

APPARATUS

The body of the pycnometer and the expansion chamber were turned from brass bar stock. The plug threaded into the body at the lower end was of the same material, as was the plate at the top of the expansion chamber, which was secured with three screws.

The Hoke-type needle valves were threaded into the body of the pycnometer and the upper plate of the expansion chamber. Adapters (not shown in Figure 1) were threaded onto the valve exits for use with flare-type fittings. All the metal-to-metal joints were soldered.

The reading tube was a section of borosilicate glass capillary tubing, $^5/_{16}$ inch in outside diameter and $^1/_{16}$ inch in inside diameter, which had been graduated at 2-mm. intervals over a span of 5 cm. The ends of the tube were ground down flat, using a drill-press and corundum powder. The tube was sealed in place with Teflon gaskets by mechanical pressure from three bolts extending from the body of the pycnometer through the expansion chamber assembly.

Two pycnometers of this type were constructed, with capacities of ca. 36 and 40 ml. The capacity of the expansion chambers was ca. 3 ml. The tare weights of the two pycnometers were approximately 500 and 700 grams.

CALIBRATION

The pycnometers were calibrated with water at 0° C., the temperature at which all samples were taken.

The pycnometer was cleaned thoroughly with acetone, followed by an alcohol rinse, and evacuated with a Hyvac pump. The outside was then cleaned with acetone, and the pycnometer was allowed to come to constant weight. It was then connected at the lower valve to the container of water by means of an L-shaped length of copper tubing.

The liquid containers used (bombs), were of the small (500-ml.) aerosol type, fitted with Hoke-type valves and adapters for flare fittings. The blow-out plugs could be removed for cleaning purposes, or to furnish an air inlet if the contained liquid had too low vapor pressure to force it from the bomb.

The pycnometer was then immersed in an ice-water bath, so that the water level came to a mark on the stem 1 cm. above the body of the pycnometer. The lower valve was opened by means of a specially constructed long-handled wrench, and the system was evacuated through the top valve.

Both valves were closed, and the vacuum line was disconnected. The bomb valve was then opened, and the pycnometer was allowed to fill to the reference mark, the flow being controlled with the pycnometer valve. About 15 to 20 minutes were usually required for temperature equilibrium.

The pycnometer was removed from the ice bath and disconnected, and the outside was thoroughly washed with acetone and then allowed to come to constant weight. After each calibration run the pycnometer was again immersed in the ice bath. In each case the liquid level came back to the reference mark.

The results are shown in Table I.

Table I. Volume of Pycnometers at 0° C.

	Pycnometer 1, Ml.	Pycnometer 2, Ml.
	39.97	36.15
	39.95	36.13
	39.94	36.15
Av.	39.95	36.14

Table II. Densities of Pure Liquids at 0° C.

Compound	Pycnometer 1	Pycnometer 2	Other Workers
CF_2Cl_2	1.402	1.401	1.395 (*2*)
	1.399	1.401	
	1.401[a]	1.399[a]	
$CHClF_2$	1.287	1.286	1.285 (calcd.) (*1*)
$CClF_2$–CCl_2F	1.622	1.621	1.6212 (calcd.) (*1*)
			1.6210 (*8*)
			1.6200 (*7, 9*)
			1.6195 (*5*)

[a] Run on refractionated material.

DENSITIES OF PURE LIQUIDS

Density determinations were made on dichlorodifluoromethane, difluoromonochloromethane, and 1,1,2-trifluoro-1,2,2-trichloroethane, using the same procedure as used in the calibration. The results summarized in Table II give a comparison of the densities obtained by this method with those obtained by other means.

No corrections were made for buoyancy, because most of the results reported are accurate to within 0.001. However, a simple buoyancy correction has been suggested by Lipkin *et al.* (*6*) where the correction is $C = 0.0012 \times (1 - \text{determined density})$. This works only where the pycnometer has been calibrated with water as recommended here.

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Method for Facilitating Recording, Filing, and Intercomparison of Infrared Spectra

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THE large recorder traces, obtained in the qualitative scanning of infrared spectra, are inconvenient to file, difficultly accessible after filing, and cumbersome to handle when simultaneous intercomparison of several records is desired. In the case of a Beckman IR-2 infrared spectrophotometer, these disadvantages may be obviated and operator attention minimized by the following procedure, which should be readily adaptable to other instruments.

The marker switch, which marks the record at intervals corresponding to each 30° of arc on the calibrated wave-length dial, is replaced with an ordinary tapping key. Using a mechanical automatic slit drive to close the slits at an appropriate rate, the 15- to 8-micron region is scanned in 23.5 minutes without attention, the 15-micron position on the chart being recorded by means of the tapping key. The 8- to 2-micron region is then scanned at a slower rate (28.5 minutes) without attention. With a recorder chart speed of 30 inches per hour, a 26-inch record is obtained. The slit shaft is driven by the idler shaft of the wave-length drive by means of a sprocket wheel and ladder chain assembly. Figure 1 shows the type of background trace obtained with this device.

Records of convenient filing size and with a permanent wave-length scale attached are then obtained as follows:

With the instrument dark (shutter closed) the 15- to 2-micron range is scanned exactly as described above and the

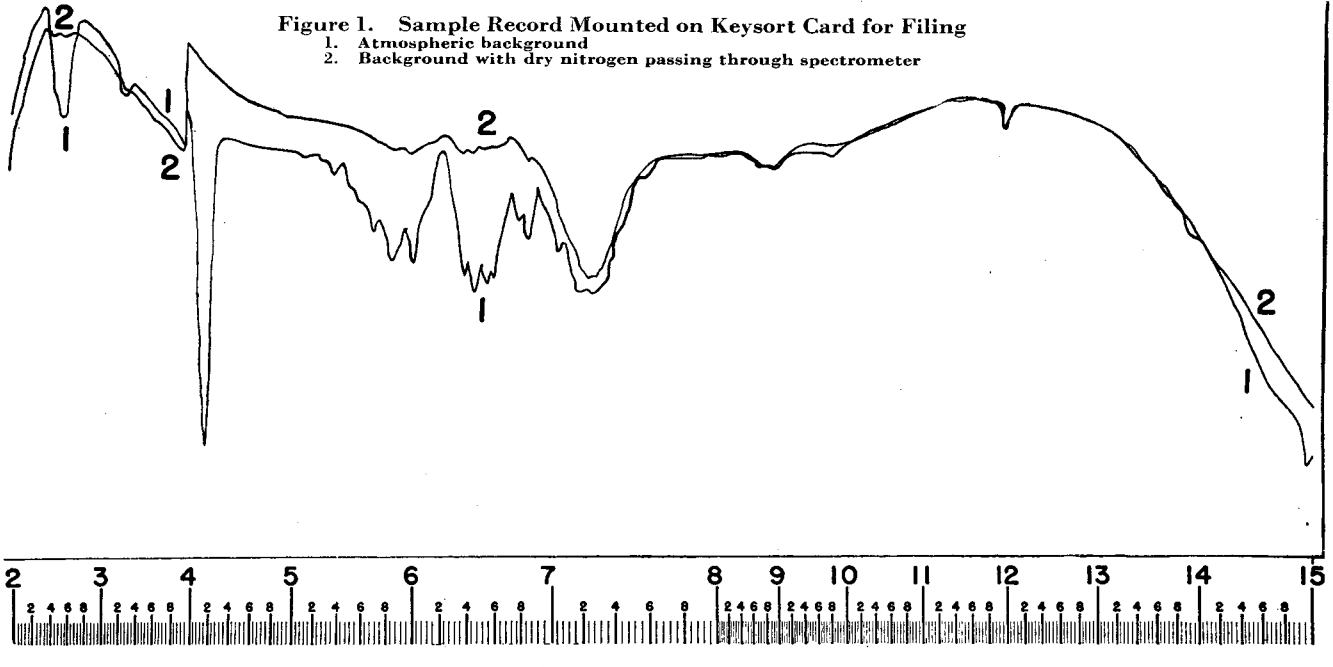

Figure 1. Sample Record Mounted on Keysort Card for Filing
1. Atmospheric background
2. Background with dry nitrogen passing through spectrometer

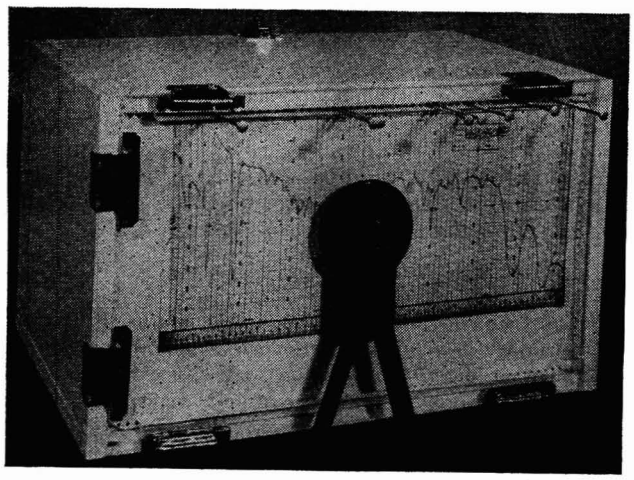

Figure 2. Sorting Box for Keysort Cards

tapping key is tapped every 0.04 micron—i.e., as every other calibration mark on the wave-length dial crosses the hairline. (Marking at 0.02-micron intervals results in poor delineation in some spectral regions.) When the calibration marks have been transferred to a strip of record paper in this fashion, a wave-length scale applicable to all records run by the standardized scanning procedure is easily constructed. This scale is permanently attached to each record by aligning the 15-micron mark on the scale with that on the record and photostatically reducing the combination to half size (13 × 5.5 inches). Wave lengths can readily be read from these reduced records to 0.04 micron in unfavorable regions of the spectrum and to 0.02 micron in more favorable regions.

The reduced records (Figure 1) are mounted on 8.5 × 15 inch Keysort cards (McBee Company, Athens, Ohio) and punched for key absorption bands at the top and chemical classification at the bottom. Provision for punching record number along the right-hand edge makes possible the selection of any record, with the aid of an alphabetical card index, and precludes the necessity of keeping the file in any particular order.

The cards, which are rather large for hand sorting, are sorted with the aid of a specially designed sorting box (Figure 2) which can be rotated 90° or 180° to permit either of the three types of sorting indicated above. The front of the box is made of transparent Plexiglas and the top, bottom, and one side are hinged to permit dropping of cards after insertion of needles through the appropriate parallel slots at front and back.

If adequate photostating service is available, the entire procedure soon becomes a routine standard practice which greatly enhances the utility of an ever-increasing infrared library in day-to-day analytical work.

ACKNOWLEDGMENT

The simple slit drive arrangement now in use was first suggested to the authors by W. C. Kenyon, Experimental Station, Hercules Powder Company, Wilmington, Del.

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Improved Centrifuge Type of Ultrafiltration Apparatus

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THE first use of centrifugal force to obtain ultrafiltration pressure was described by de Waard (*7*) in 1918. A much improved technique was described by Rehberg (*5*), but the author's previous experience indicated that the membrane support of metal would probably introduce adsorption errors. In addition, specially made cellophane tubes were required.

In a previous ultrafiltration study (*1*) of beryllium solutions of very low concentrations, quantitative interpretations were impossible because of adsorption losses on the apparatus employed. An improved apparatus of the centrifugation type has been devised which gives excellent reproducibility and an absolute error of about 6% and embodies the separate advantages of those previously described (*2, 4, 5, 7*). The new apparatus is of all-glass construction and employs the readily available Visking seamless cellophane tubing as the membrane.

APPARATUS

The upper part of the apparatus, shown in Figure 1,*a*, was made by sealing the ball part of an 18/9 ball-and-socket joint to a coarse porosity, borosilicate glass fritted sealing tube, 25 mm. in diameter. The socket part of the joint connected to a bulb of about 4-ml. capacity served as the receiver for ultrafiltrate.

A cellophane bag, Figure 1,*b*, made by tying double knots in the ends of a piece of Visking seamless cellophane dialyzing tubing, 19 mm. in diameter, was used as the container of the solution to be filtered. This was fitted into the upper part of the apparatus as shown in Figure 1,*c*.

To keep contaminating dust out of the apparatus during the centrifugation, the top of the ultrafilter was covered with a piece of cellophane and sealed thereto with Scotch tape. Scotch tape and fabric-covered wire were used to seal the ball joint. The ultrafilter was wrapped with a sheet of 0.125-inch (0.3-cm.) sponge rubber to make it fit snugly in the metal centrifuge tube.

In an International centrifuge, size 2, eight ultrafiltrations of 25 ml. each can be run simultaneously. Temperature-controlled experiments can be performed, four at a time, in the International Portable Model PR-1 refrigerated centrifuge.

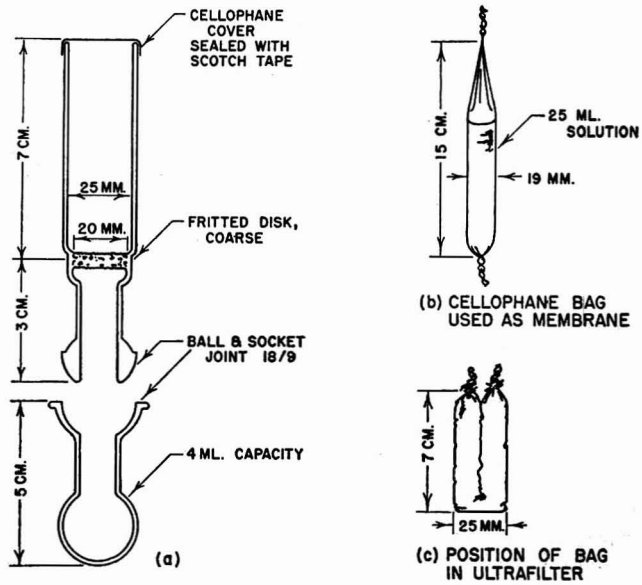

Figure 1. Apparatus

METHODS

Solutions of beryllium were prepared by dilution of a stock solution (*6*). Suspensions of beryllium hydroxide (Table I) were prepared by the addition of 5 ml. of 0.125 *M* sodium bicarbonate to each 20 ml. of the appropriate solution of beryllium. The resulting suspensions were allowed to stand 2 hours before ultrafiltration. It is understood that these suspensions were not pure beryllium hydroxide, but rather a mixture of beryllium hydroxide and basic carbonates.

Table I. Ultrafiltration of Colloids

Substance Filtered[a]	Centrifugation Rate/1000	Centrifugation Time	Ultrafiltrate Volume	Ultrafiltrate Colloid concentration
	R.p.m.	*Hours*	*Ml.*	*% of initial concn.*
$Be(OH)_2$ (4.1γ/ml.)	1	3	2.5	1.0, 0.3
$Be(OH)_2$ (1.6γ/ml.)	1	3	2.5	0, 0.2
$Be(OH)_2$ (4.1γ/ml.)	2	1	2.2	0.2
$Be(OH)_2$ (1.6γ/ml.)	2	1	2.2	0.2
$Be(OH)_2$ (4.1γ/ml.)	2	2	4	0.5
$Be(OH)_2$ (1.6γ/ml.)	2	2	5	1.2
Protein (fresh blood plasma)	1	6	2.5	0,0
	2	2	2.5	0,0
Protein (Difco serum)	1	6	2.5	0,0
	2	2	1	0,1
Inulin (3.25 mg. %)	1	3	2.2	68, 68, 92

[a] Tracer method of analysis used for all $Be(OH)_2$ suspensions.

Plasma was prepared by centrifuging oxalated beef blood obtained at a local slaughterhouse. The 10% Difco serum suspensions were prepared from Difco Bacto dried beef blood serum. All other chemicals used were C.P. grade.

Beryllium was determined either by the colorimetric method (*6*) or the tracer technique (*1*). Protein was determined by visual comparison of the turbidity obtained upon the addition of 3 drops of 5% sulfosalicylic acid to the test solution and to known dilutions of plasma. Inulin was determined by the method of Kruhoffer (*3*).

RESULTS

The force and rate of ultrafiltration are, of course, related to the speed and radius of centrifugation and also are dependent upon the solution being centrifuged. Thus, at 1000 r.p.m., 2.5 ml. of filtrate were obtained from aqueous solution in 3 hours and from blood plasma in 6 hours. At 2000 r.p.m., the time required for the collection of 2.5 ml. of ultrafiltrate was 1 and 2 hours for aqueous solutions and blood plasma, respectively. The filtration of solutions of Difco dried serum was very slow, probably because of clogging of the membrane. These data are presented in Table I.

Table I also shows that the membrane is permeable to small colloidal particles such as inulin but impermeable to beryllium hydroxide and to protein. For this work, such dilute suspensions of inulin were required that the probable error of the analyses was rather large. Accordingly, the values reported for inulin are of qualitative significance only.

When solutions of beryllium, in concentrations ranging from a trace to 4.64 micrograms per ml. in 0.001 *M* hydrochloric acid, were centrifuged at 1000 r.p.m. for 6 hours, the concentration of beryllium (94 ± 1%) in the filtrate was equal to that remaining in the cellophane bag. The consistently low recovery probably is due to a slight adsorption on the cellophane bag.

DISCUSSION

The all-glass construction of the new apparatus represents a real advantage in eliminating problems of adsorption and decontamination, permitting accurate studies of extremely dilute solutions. Furthermore, the temperature at which the filtration is carried out may be conveniently controlled. In spite of a consistently negative absolute error of about 5% (with low concentrations of an adsorbable solute) the ultrafiltration technique described gave very reproducible results. The accuracy seems adequate to permit quantitative studies.

Under the experimental conditions employed, the membrane was permeable to dissolved beryllium and a low-molecular weight colloid, inulin, but impermeable to protein and precipitated beryllium hydroxide.

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Improved Manostat and Manometer

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ALTHOUGH many pressure controllers suitable for use in vacuum distillations have been reported (*5*), it is believed that the manostat described here is better adapted to precision vacuum fractionation than those previously described. A novel feature is the use of a completely enclosed magnet-operated screw, which permits rapid and precise adjustment to any pressure over a wide operating range. The manostat is substantially independent of small variations in room temperature or pump capacity. Moreover, it is small and light in weight, and may be clamped conveniently to the usual laboratory rack or ring stand. Its construction requires the services of a skillful glassblower. A manufacturer of scientific apparatus is now developing a model for commercial production. Because deviations from the controlled pressure depend not only on the pressure-sensitive element, but also on the rest of the system, Figure 1 gives a semischematic representation of the arrangement used to obtain the degree of constancy reported here.

The modified 40 × 170 mm. borosilicate glass test tube, 13, has an 8-mm. sidearm, 10, and a 14/38 ground-glass neck, 9. It holds approximately 40 ml. of mercury. The amount is not critical, but should be enough to fill the central tube completely, in case tube 13 is allowed to come to atmospheric pressure.

The 8-mm. central tube, 12 (soft glass ground to size 14/38), extends to within a few millimeters of the bottom of tube 13 and terminates in a 0.5-mm. hole, 15. The upper portion, 12 × 160 mm., contains two glass tabs, which hold stainless steel bracket 7; this bears two extensions, 25 and 8; 25 serves as a bearing for the stainless steel screw, 4 (50 threads per inch), and 8 serves as pivot for the screw. The upper end of the screw terminates in a soft iron vane, 3. On the screw is threaded stainless steel nut 5, of such size that when the screw is turned the nut is prevented from turning with it by the bracket, and so must advance or retreat along the screw. Attached to nut 5 is a stiff tungsten wire lead, 11, which is connected electrically to the outside of the central tube at the top through the nut, screw, bracket, and platinum wire 27, which is sealed through the tube.

The central tube also contains the platinum lead, 14, which is in contact with the mercury at all times and is held against the side by a thin glass tube tacked to the wall. This lead is also sealed through at the top, thus making the second of terminals 1, 1

The seals at the top are protected by thermoplastic cement, which also holds in place the metal guide, 26, on which rests the annular magnet, 2. As the magnet is turned, it turns vane 3 and thus screw 4, moving the tip of the tungsten electrode axially within the tube.

The terminals are connected to an electronic relay, 28, which operates solenoid 22 (1000 ohms). Use of an electronic relay minimizes the probability of sparking at the contact between

electrode 11 and the mercury. A spark is undesirable, especially where flammable materials or explosive vapors may be present in the confined space.

The solenoid is connected to the relay, so that when the manostat circuit is broken, plunger 23 (faced off with a soft rubber pad, 24) is drawn up, thus opening orifice 16.

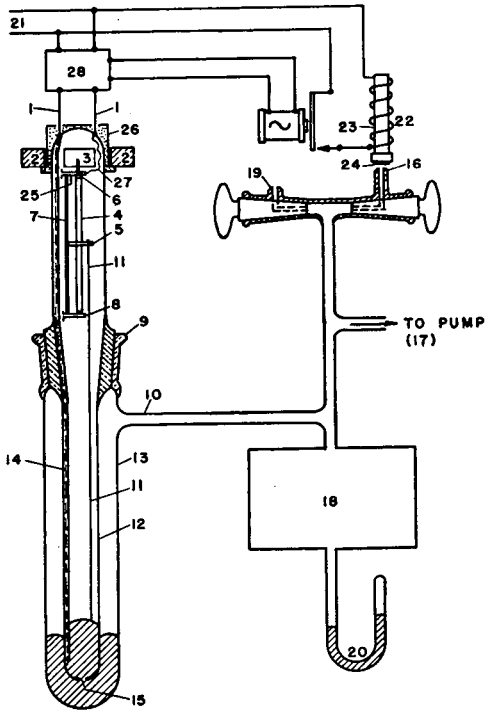

Figure 1. Diagram of Manostat

The flow of air into the system and pump is controlled by a special stopcock, with independent adjustment of a continuous leak through orifice 19, and adjustment of the intermittent leak through orifice 16. The plug of each stopcock is carefully grooved to permit throttling of gas flow. Orifice 16 is a 1-mm. capillary ground to a conical shape and then ground flat on the sealing surface.

To place in operation, side arm 10 is connected with pressure tubing to a good pump, and the manostat is tilted to expose hole 15 and thoroughly evacuated. The manostat is then restored to the upright position; the side arm is clamped off, detached from the pump, and attached to the system to be controlled, 18.

Pump 17 is started, and with orifice 16 closed by the stopcock, the system is pumped to a few millimeters below the desired pressure by adjustment of orifice 19. At the lower pressures, orifice 19 is completely closed. The pinchclamp on the manostat is next opened, and the position of electrode 11 is adjusted to open the circuit and lift the plunger. The stopcock on the right is then opened slightly to permit the pressure to rise slowly. As soon as the mercury touches electrode 11, the relay permits the plunger to fall on orifice 16. The pressure begins to fall and the mercury is withdrawn from contact 11, whereupon the plunger rises, and the cycle repeats.

With proper adjustment of the stopcocks, operation will occur with a slight "breathing" motion of the mercury meniscus. If the equilibrium pressure in the system, measured, for example, by manometer 20, is not that desired, it is quickly brought to the correct point by turning the magnet (one complete turn of the screw advances the nut 0.5 mm.). Adjustments can be made to a more precise degree than can be read on the usual U-tube manometer.

The manostat prepared in this way is also an absolute manometer. For some purposes, measurement by this manometer may be sufficient, but it is usually desirable to connect a second manometer directly to the system. When the manostat is to be used as a manometer, a millimeter scale is fastened to the side of the tube, or may be etched directly on tube 13.

If it is necessary to control pressures higher than about 100 mm., the manostat is tilted on its side, and air is admitted to the central tube while the system is near the desired pressure. If air at 100 mm. is admitted, the new operating range is 100 to 220 mm.; if air at 220 mm. is admitted, the range is 220 to 375. The range 3 to 760 mm. can be covered in five steps. Explosive vapors should be excluded from the central tube, even though the use of an electronic relay minimizes the probability of sparking.

It is preferable to connect the manostat and leak by a T-type connection near the pump. With an ordinary distilling column no surge chamber between the system and manostat connection is necessary, but if the controlled system has a small volume or if the pressure is high (150 mm.), a surge chamber will help smooth out the pressure variations caused by the controlling action.

It is unnecessary to clamp off the manostat when fractions are changed; admission of air into tube 13, as in evacuating a fresh receiver, causes no permanent change in the system; controlling action resumes when the mercury level reaches the end of the electrode.

In some cases of this kind, however, cohesion of the mercury and glass results in a temporary control point 0.1 to 0.2 mm. away from the initial pressure, to which the controller readjusts rather slowly. Hence, it may be advisable to tap the manostat or make an adjustment.

The manostat retains all the advantages of an adjustable electrode (*1*), but avoids the uncertainties and difficulties of leakproof packing (*2*); moreover, the controlling action in the range 3 to 100 mm. occurs in a vacuum, where fouling of the mercury is less likely to occur.

Because the manostat and relay, in effect, constitute a switch, modifications in the leak system could be made to meet various requirements. The relay could be used to switch a pump (*2*, *7*), operate a breather valve between system and pump (*6*), or bleed inert gas to a system by means of a breather valve (*4*).

The authors have used the manostat to control the distillation pressures for a series of boiling point measurements (*3*), and to check reported boiling point measurements. The lowest pressure at which it was operated was 3.0 mm. Orifice 19 was closed off entirely, and after preliminary adjustment the pressure remained constant for several hours, without any visible variation in pressure apparent on a Dubrovin gage, which is calibrated in 0.2-mm. intervals.

At 144.0 mm., the pressure remained constant within ±0.1 mm. for 5 hours. The highest pressure at which the manostat was operated was 523 mm.

At the highest pressure, the capacity of the pump exceeded that of the leaks, and it was necessary to throttle the pump with a pinchclamp. The test was run for 3 hours; the pressure remained at 523 ± 0.2 mm.

ACKNOWLEDGMENT

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Pipet Method of Sedimentation Analysis

Rapid Determination of Distribution of Particle Size

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THE pipet method of sedimentation analysis is well known (*1–5*). In a critical study of sedimentation methods Schweyer (*6*, *7*) and Work (*8*) showed the advantages of Andreasen's pipet method (*1*, *2*). Schweyer attempted (*6*) to reduce the duration of experiment by using a special kind of tap, but the equipment has to be specifically made, it involves complicated glass blowing, the depths at which the pipets can be immersed in the suspension cannot be altered at will, and the pipets cannot be operated simultaneously. In the change-over from one pipet to another, the distribution function of the particles that have passed the tip of the shorter pipet and still lie above the tip of the longer pipet cannot be determined with certainty.

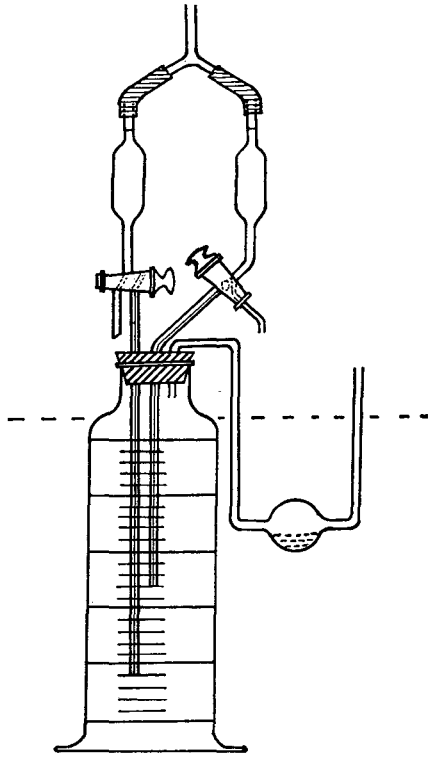

Figure 1. Graduate

The present work was undertaken to minimize the time required. The apparatus was designed to withdraw samples at two different depths simultaneously and not consecutively as was done by Schweyer.

Theoretically, the whole size range may be obtained by analysis of samples at different depths at one and the same time, but experimental difficulties prevent such a determination. However, by using two or three pipets operating simultaneously at two or three different depths, the time of observations can be considerably shortened. The time necessary for the operation of the experiment will obviously depend on the smallest particle size desired.

EXPERIMENTAL

A 1000-ml. cylindrical graduate (Figure 1) is fitted with a rubber bung with three holes. Two capillary tubes pass through two of the holes into the suspension in the cylindrical graduate at two different predetermined depths. The capillaries are joined to two independent 10-ml. pipets by means of three-way taps. The tops of the two pipets are connected to a Y-tube, by means of which samples can be withdrawn simultaneously. Through the third hole, a bent tube with a bulb containing some water (medium) immersed in the thermostatic bath is fitted to minimize evaporation losses. The experimental procedure is as usual. China clay passing through a 200-mesh screen (Tyler) was used in water throughout the investigation.

Tables I and II show the results of a single-pipet experiment in comparison with the double-pipet method.

Table I. Experiment with Single Pipet of Andreasen Type

Time, Min.	Particle Size (Diameter, Microns)	% Undersize
3	25.54	97.31
10	13.87	94.91
30	6.92	82.04
60	5.57	73.98
120	3.90	60.22
210	2.93	45.71
275	2.54	37.72
515	1.82	25.77
1130	1.23	14.79

Table II. Experiment with Double Pipet

Time Min.	Particle Size (Diameter, Microns)		% Undersize	
	Long pipet	Short pipet	Long pipet	Short pipet
	A, First Experiment			
4.75	20.41	16.03	98.35	88.74
11	13.18	...	93.99	...
35	7.25	5.56	73.21	70.49
60	...	4.14	...	56.54
120	...	2.85	...	43.46
240	2.82	1.95	36.76	26.61
360	2.11	1.54	23.33	20.81
480	1.80	1.28	22.14	15.65
	B, Duplicate Experiment			
3	25.47	19.91	95.28	80.19
10	13.71	...	90.42	...
30	7.78	5.94	82.22	67.19
60	5.42	4.09	65.09	58.51
125	4.13	2.74	55.94	38.95
240	2.61	1.91	36.04	23.57
360	2.09	...	26.57	...
480	1.78	1.26	21.10	16.63

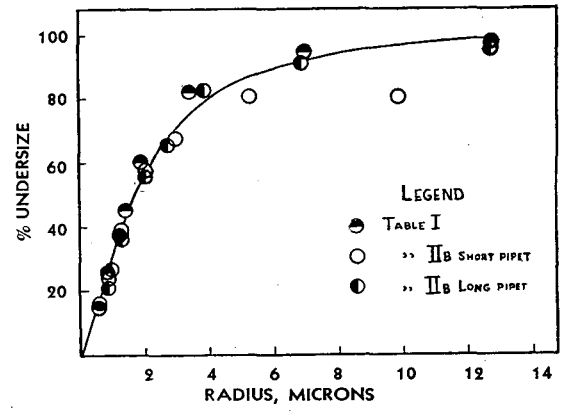

Figure 2

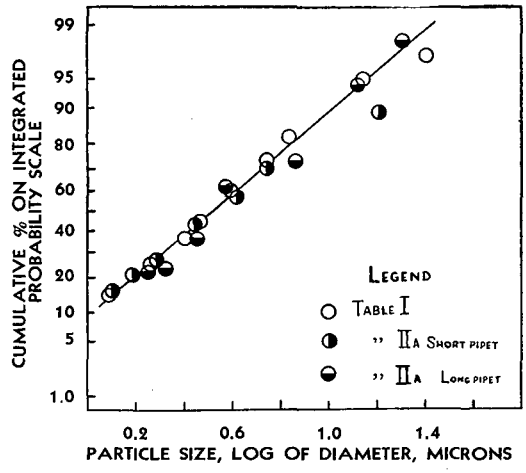

Figure 3

DISCUSSION

In Figure 2, the results in Table I have been plotted with those of Table II,B, to give a common cumulative percentage curve. The duplicate experiment with double pipet was made to indicate that the agreement between the single pipet and the proposed double-pipet method is decisive and not accidental.

As a further test of validity and reproducibility of this method, Austin's (*3*) suggestion of plotting particle size on a logarithmic scale and cumulative percentages on an integrated probability scale has been adopted in Figure 3. The log-probability graph is linear, as anticipated. However, a point or two at the commencement of the experiment is off the linear graph. This may be due to inaccuracy in recording the zero time and initial sedimentation.

The time of 1130 minutes required for obtaining the per cent undersize of particles of 1.23 μ diameter with a single pipet (Andreasen type) has been reduced to 480 minutes to obtain nearly the same particle size. Obviously, the saving of time effected depends upon the depth at which the short pipet is located.

The apparatus is easily assembled.

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Infrared Absorption Band of *n*-Butyl Group

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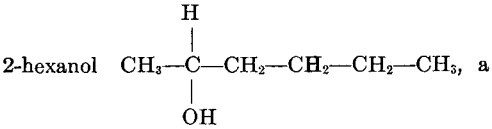
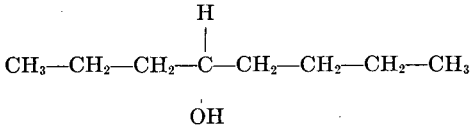
IN RUNNING the infrared spectra of a series of ethers it was noted that all the ethers containing an *n*-butyl group showed a strong absorption band at approximately 739 cm.$^{-1}$ which was lacking in the other ethers.

This band is in the region of 720 to 760 cm.$^{-1}$, suggested by Sheppard and Sutherland (*2*, *3*) as being a reasonable range within which one can expect the occurrence of the CH_2 rocking modes. In further agreement with the assignment of this absorption to the CH_2 rocking mode, Vallance-Jones and Sutherland (*7*) showed by use of polarized infrared radiation on an orientated polyethylene sample that the change in electric moment occurring during this vibration at approximately 720 cm.$^{-1}$ was perpendicular to the carbon chain. Sheppard and Sutherland (*3*) state that any molecule containing $(CH_2)_nCH_3$, in which n is equal to or greater than 3, possesses a band in this region of 720 to 760 cm.$^{-1}$ This would include the *n*-butyl group.

Tuot and Lecomte (*6*) have reported that, in a study of the infrared spectra between 700 and 800 cm.$^{-1}$ of a series of approximately 30 alcohols, indications can be obtained regarding the length and branching of the carbon chain. They find for alcohols, in which the *n*-butyl group is the longest unbroken chain present without substitutions, a band at approximately 274 cm.$^{-1}$—for example, for 2-hexanol



maximum absorption band at 724 cm.$^{-1}$ However, they also report for 4-octanol



a maximum absorption band at 735 cm.$^{-1}$

Recent articles by Barnes, Gore, Stafford, and Williams (*1*) and by Thompson (*5*) contain tables listing functional group absorption frequencies but do not specifically include the *n*-butyl group. From the chart in Thompson's article the following approximate ranges can be obtained.

		Cm.$^{-1}$ (Estimated from Chart)
One methylene group	$CH_3—CH_2—$	760–780
Two methylene groups	$CH_3—CH_2—CH_2—$	730–750
Four methylene groups	$—CH_2—CH_2—CH_2—CH_2—$	715–730

The information on these three groups is based on work done on the spectra of hydrocarbons at Oxford and Cambridge.

Because the work of Sheppard also referred to hydrocarbons and that of Tuot and Lecomte to alcohols, it was considered worth while to investigate the position of this band in various types of organic compounds containing the *n*-butyl group. It is of qualitative value to establish the approximate location of the *n*-butyl group and the variation in the range of frequency that may be expected for a given series of *n*-butyl compounds.

EXPERIMENTAL PROCEDURES

The organic compounds used were Eastman Kodak (White label grade), except for diethyl Cellosolve, butyl Cellosolve, dibutyl Cellosolve, and dibutyl Carbitol, which were obtained from the Carbide and Carbon Chemicals Corporation. The compounds were purified by distillation immediately prior to use and a sample boiling within ± 0.1° C. of the literature value was used for the absorption measurement. In the case of the ethers and alcohols, distillation over calcium hydride was employed to remove all traces of water, inasmuch as sodium chloride windows were used in the 0.025-mm. sample holder.

The measurements were made with a Perkin-Elmer infrared recording spectrometer Model 12B, using a sodium chloride prism. The prism was calibrated with ammonia gas, carbon dioxide vapor, atmospheric water vapor, and benzene vapor using a 10-cm. gas cell.

Table I. Absorption Bands

Compound	Frequency, Cm.$^{-1}$	Compound	Frequency, Cm.$^{-1}$
In *n*-Butyl Compounds			
Ethers		Amides	
n-Butyl	739	Di-*n*-butylcyanamide	736
Dibutyl Carbitol	739		
Butyl Cellosolve	738	Amines	
Dibutyl Cellosolve	739	Di-*n*-butylamine	737
		Tributylamine	734
Esters		*n*-Butylaniline	740
n-Butyl acetate	739		
n-Butyl lactate	739	Nitriles	
n-Butyl propionate	739	*n*-Valeronitrile	740
Di-*n*-butyl oxalate	740		
Di-*n*-butyl tartrate	740	Halides	
Di-*n*-butyl succinate	740	*n*-Butyl bromide	742
		n-Butyl iodide	736
Alcohols			
n-Butyl	738		
In Compounds without *n*-Buty]Group in Region of 720–750 cm.$^{-1}$			
Ethers		Esters	
Diethyl	None	*n*-Propyl acetate	721, 759
Diethyl Cellosolve	None	*n*-Amyl acetate	721
Isopropyl	None		
		Amines	
Alcohols		Diethylamine	None
Methyl	None		
Ethyl	None	Halides	
n-Propyl	753	*n*-Propyl bromide	740
n-Amyl	730		
Isobutyl	None		
tert-Amyl	727		

DATA AND DISCUSSION

Eighteen available compounds of varying types, all containing one or more *n*-butyl groups, were measured over the region of 720 to 750 cm.$^{-1}$

To show that the appearance of a band at approximately 739 cm.$^{-1}$ is reasonably indicative of the presence of the *n*-butyl group in the compounds under consideration, a few alcohols, esters, ethers, amines, and halides not containing an *n*-butyl group were also run in this narrow region. Table I lists the results obtained.

The data present strong evidence that an absorption band in the region 734 to 742 cm.$^{-1}$ is indicative of the presence of the *n*-butyl group, in the compounds listed. This band is of moderate intensity, being slightly stronger in compounds containing two butyl groups as compared to those containing only one. It is especially significant in the case of the ethers and esters.

Smith (*4*) has pointed out the following, based on a survey of the first 700 of the 800 spectrograms of the A.P.I. series.

Of these, 220 had a band between 725 and 750 cm.$^{-1}$, and 34 of the 220 had a band of medium or strong intensity between 734 and 742 cm.$^{-1}$ Of the 34, only 6 contained *n*-butyl groups; 17 had *n*-propyl groups, and the remainder were a variety of compounds such as toluene, 1,2-dimethylbenzene, *n*-hexadecylbenzene, hexachlorothiolane, 1,2-dicyclohexylethane, 2-acetyl-3-methylthiophene, β-methylstyrene, and 2,2,4-trimethylhexane. Six compounds containing *n*-butyl groups had strong bands from 728 to 730 cm.$^{-1}$ If the range is broadened to include them, such compounds as 1-heptene, 2,3-dimethylbutane, 3-ethylpentane, and 1-cyclohexyl-3-cyclopentylpropane also will be included. A similar situation exists at the upper end of the range above 742 cm.$^{-1}$

That the *n*-propyl and *n*-amyl compounds have absorption bands in this region is also to be expected from the references previously cited. In Table I the presence of the *n*-propyl bromide absorption band at 740 cm.$^{-1}$ illustrates the fact that an absorption band between 734 and 742 cm.$^{-1}$ indicates only that the *n*-butyl group may be present and by no means constitutes conclusive evidence, because, as further emphasized by Smith, this region is overlapped by the propyl group.

All the *n*-butyl compounds used in this study except for *n*-valeronitrile have the *n*-butyl group separated from the rest of the molecule by a functional group containing either oxygen or nitrogen. In such cases the absorption band attributed to the *n*-butyl group would be expected to be more specific than in a group that is simply a part of a long straight or branched carbon chain, as in the case of the hydrocarbons mentioned by Smith or the alcohols studied by Lecomte. It would seem, therefore, that this absorption band in the region 734 to 742 cm.$^{-1}$ should be restricted to compounds containing *n*-butyl groups attached to a functional group.

Moreover, this absorption band for the above butyl compounds which contain three methylene groups fits well with the previously cited data of Thompson.

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Comparison of Melting Point Methods for Wax

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THERE are numerous methods for the determination of the "melting point" of waxes. Many of these methods do not agree on the melting point value of the same wax, and many are affected by the type of wax. For this reason an investigation has been made of a number of melting point determination methods as applied to various waxes, ranging from the paraffins of low melting point, through the plastic microcrystalline and high melting paraffins or microcrystalline waxes, and including three representative oxidized hydrocarbon waxes. The purpose was not primarily a comparison of methods on the basis of their individual reproducibility, but rather a comparison of the average melting points as shown by them. The softer, lower melting point microcrystalline waxes of the type obtained from lubricating oil residue are referred to as "plastic microcrystalline."

The methods used were those most generally encountered in the trade. They are listed below with an abstracted outline of procedure and a reference to the more complete description in the literature.

METHODS INVESTIGATED

Melting Point of Petrolatum. Briefly, this method (*2*) consists of lightly coating the bulb of a special A.S.T.M. thermometer by dipping it into the wax. The thermometer is then placed in an air bath on which the temperature is raised at a specified rate of 2° F. per minute until the wax drips from the thermometer.

Melting Point of Paraffin Wax (*1*). A molten sample of wax is placed in a specified apparatus consisting of a tubular container together with a thermometer and stirring device. The container is then placed in an air bath so controlled as to permit lowering of the temperature at a specific rate. Time *vs.* temperature read-

ings are plotted and the melting point is determined as that point at which the rate curves indicate a minimum rate of temperature change with time.

Continental Solid Point (Galician) Method (6). The bulb of a standard 1° F. division thermometer is dipped into melted wax at about 20° F. above its melting point. If the thermometer is held in the hands at an angle of about 15° above the horizontal, a drop of moltex wax is formed on the lower end of the bulb. The thermometer is then slowly rotated on its axis and a reading of the temperature is taken at the point at which the drop solidifies and rolls to the top of the thermometer. (This method is in very general usage in Continental Europe.)

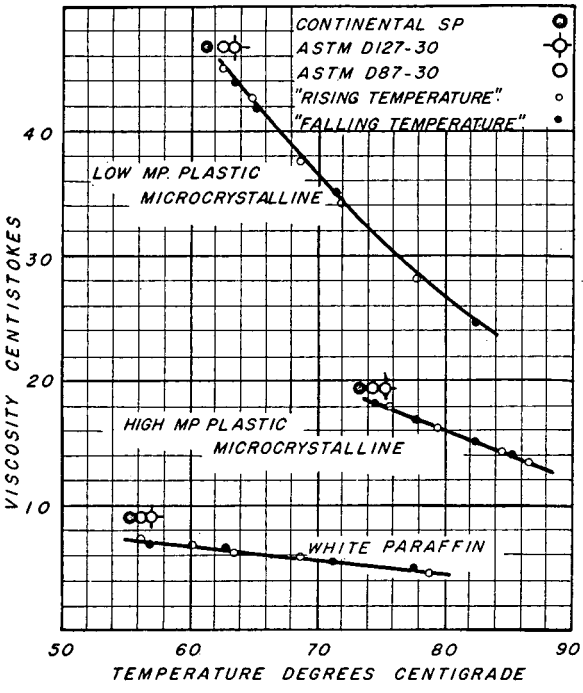

Figure 1. Viscosity-Temperature Relationship near Melting Point for Crystalline Paraffin and Plastic Microcrystalline Waxes

Test of Softening Point. This is the well known asphalt softening point test (4), included here merely because it is used by some wax users. The tentative (shouldered ring) method was used because the wax does not adhere to the form ring as does asphalt. The method consists of molding a sample of material into a specified bottomless ring, attaching it to a thermometer, and weighting the top surface of the sample with a steel ball. The assembly is then placed in a fluid bath (usually water on waxes) and the temperature is raised at a specified rate of 9° F. per minute. The softening point is the temperature at which the ball drops through the sample to the bottom of the bath.

Fisher-Johns Melting Point Block Method. This is an electrical micro melting block of aluminum with a magnifying glass attachment for viewing the sample (8). About 0.1 mg. of finely ground wax is placed between two microscope cover glasses on the heating block. The temperature is raised at about 3° F. per minute until the wax is seen to be liquid.

Crown Cork and Seal Ring and Plunger Method. This is essentially the same as the A.S.T.M. ring and ball method (8), except that a weighted pointed plunger is set in a guide frame on the surface of the wax sample. The weight of the plunger is much greater than that of the ball in the A.S.T.M. test. Procedure is practically the same, the temperature at which the plunger sinks through the wax being taken as the softening point.

Solidification Point of Waxes. This method is similar to (1), except that the melted wax and the surrounding water bath are maintained at as nearly the same temperature as possible, and there is no stirring. The point at which the temperature-time curve flattens is called the solidification point.

U. S. Army Ordnance Method. In this method (1) a capillary tube 4 mm. in diameter and 7.25 cm. (3 inches) long is filled on one end to a depth of 1.25 cm. (0.5 inch) with a wax plug. After the wax solidifies the tube is fastened to the bulb of a thermometer and immersed to within 0.6 cm. of the top in a water (or mercury) bath, the temperature of which is raised at 3° F. per minute until the wax plug rises because of the hydrostatic pressure. This point is called the melting point.

Closed Capillary Tube Method. This is the usual organic melting point method in which a tube 1 mm. in diameter is attached to a thermometer bulb after being filled with powdered wax (8). The thermometer and tube are immersed in a circulating water bath and the temperature is raised at 3° F. per minute until the wax is observed to be melted.

Table I. Tests on Eleven Waxes

Method No.

1. A.S.T.M. petrolatum melting point (2)
2. A.S.T.M. paraffin melting point (1)
3. Continental solid point (Galician, 6)
4. Ring and ball softening point (4)
5. Fisher melting point block
6. Crown Cork and Seal, ring and plunger
7. Solidification point (Warth)
8. Ordnance melting point (7)
9. Capillary tube melting point

Method No.	1	2	3	4	5	6	7	8	9
128 AMP paraffin	57.0	56.0	55.4	59.0	..	..	56.0	54.5	56.9
Plastic micro-crystalline waxes	63.3	62.2	61.3	63.7	..	57.7	63.0	59.5	61.8
	75.0	74.2	73.4	78.2	66.3	62.6	73.9	74.5	73.9
	76.7	76.0	76.0	76.7	..	71.8	76.7	72.8	71.7
Oxidized petroleum waxes	83.5	80.2	80.0	82.1	65.0	66.1	82.7	79.4	81.6
	83.8	83.8	80.0	86.1	75.0	69.8	82.6	80.6	82.7
	87.6	85.0	84.5	84.4	81.2	67.8	85.0	82.8	81.6
Hard high melting micro-crystalline waxes	87.6	87.0	83.8	90.2	81.5	78.3	85.7	85.0	84.4
	88.4	87.8	85.5	90.2	85.0	81.2	95.8	86.4	87.5
	92.7	88.8	86.0	92.8	81.0	77.1	96.6	85.6	87.8
	93.8	90.3	86.0	95.5	87.0	83.5	97.0	85.0	86.0

Eleven different waxes were examined by each of these methods: crystalline paraffins, plastic microcrystalline, oxidizec, and hard, high melting microcrystalline. Tests were run to a minimum of two 0.5° F. checks on each wax by each method and the average values are shown in Table I.

1. A.S.T.M. petrolatum (2) and A.S.T.M. ring and ball (4) methods give consistently higher values than all other methods.
2. A.S.T.M. paraffin melting point (1), closed capillary, Warth solidification point, and the Continental solid point methods are in fairly good agreement at a somewhat lower value.
3. The U. S. Army Ordnance method (7) agrees with the methods listed above (in 2) in some cases but seems to be very erratic.
4. The Fisher melting block and the Crown Cork and Seal methods are somewhat comparable at the lowest level of all, and the divergence of these two from the general average of the other methods is greater for the plastic microcrystalline and oxidized waxes than for either the crystalline paraffins or the hard microcrystallines.
5. Variation between the different methods is at a maximum on the hard microcrystalline waxes and may range from 2° to 10° F., depending upon the method used.

The wide divergence between A.S.T.M. petrolatum melting point and the other methods in the case of the hard microcrystalline waxes appeared to be caused by the apparently high viscosity of the wax very close to the melting point. Frequently a wax was visually fluid for as much as 5° to 10° F. before a drop fell from the thermometer. This effect was not noted in the case of the plastic microcrystalline and the crystalline paraffin waxes.

To clarify this effect viscosities of a number of waxes were run in the modified Ostwald viscometer (3, Method B) at temperatures very close to the melting point. Viscosities were run on the waxes on both "rising" and "falling" temperature variations—that is, one set of values (rising) was taken as the bath tempera-

ture was raised by small temperature increments from below the melting point of the wax to temperatures considerably above the melting point of the wax; the other set of values (falling) was obtained by lowering the temperature by increments from above the melting point of the wax to the point at which the wax would no longer flow.

DISCUSSION

Results of the viscosity tests are shown in Figures 1 and 2. In the case of the crystalline paraffins and the plastic microcrystalline waxes (Figure 1) there is no pronounced rise in viscosity as the solidification point of the wax is approached from either the rising temperature or the falling temperature side, nor is there any indication of hysteresis for the crystalline and plastic waxes.

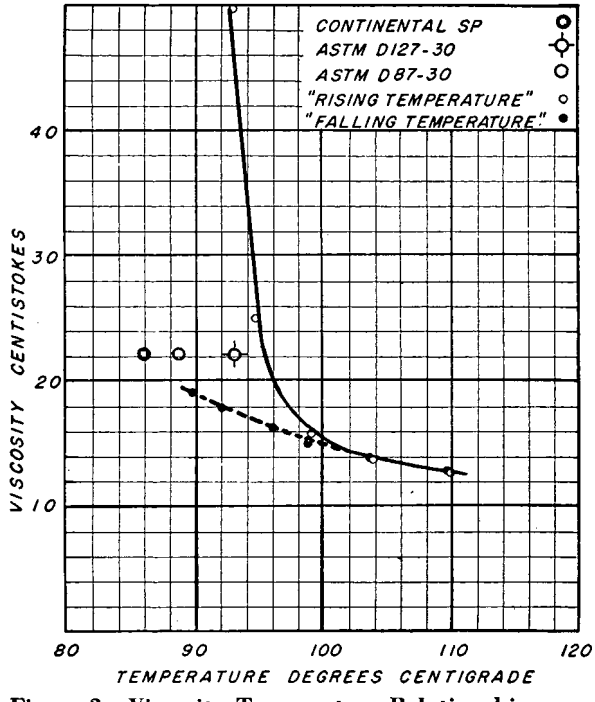

Figure 2. Viscosity-Temperature Relationship near Melting Point for Microcrystalline Waxes of High Melting Point

In the case of the hard microcrystalline waxes there is a pronounced hysteresis in viscosity on the "rising temperature" curve as may be seen in Figure 2, which is a typical member of this class. The viscosities immediately above the melting point are very high in comparison to the "falling temperature" value of the viscosity in this temperature range. These waxes, near their melting point, seem to depart from the usual viscosity characteristics of petroleum liquid. This effect definitely increases the spread between the actual melting point of the wax and the melting point as determined by the A.S.T.M. petrolatum melting point method.

The three most popular melting point methods (A.S.T.M. petrolatum, A.S.T.M. paraffin, and Continental solid point) are indicated on each of the viscosity curves. These do not show wide divergence in the case of the paraffins and plastic microcrystalline waxes. In the light of the viscosity data shown it is indicated that the A.S.T.M. paraffin melting point most nearly represents the actual melting point of the microcrystallines and is also about average for the crystalline and plastic waxes as well. This is supported by the agreement of this method with the other methods mentioned above. By extrapolation of the viscosity curves it may be seen that the actual value of the A.S.T.M. petrolatum melting point is the value of the temperature at which the viscosity of the molten wax is approximately 50 cs. rather than the true melting point of the wax. Values of the Continental solid point are uniformly low, probably because of the rapid rate of cooling, the time lag in reading the thermometer, and the lack of stem correction.

A method that merits some discussion is that of the ring and plunger (Crown Cork and Seal softening point). This method gives values which are in fair correlation with the Fisher melting point block. Readings of the melting block method in this work were taken at the first indication (visual) of liquefaction of any of the crystals. It was also noted in the course of the viscosity tests that there seemed to be a range of temperature through which the waxes melt. In some cases actual flow at very high viscosity was possible while unmelted wax crystals could be seen suspended in the partially liquefied wax. This was not observed on the falling temperature curves, however; the wax appeared to solidify instantly. It is possible that a shell formed on the outside of the viscosity tube and that in the center of the solid mass some liquid did exist. The ring and plunger method thus seems to indicate the "thaw point" or eutectic temperature (*5*) of the wax (for all these waxes are in reality multicomponent systems), and this value is approximately the true value of the "softening point" of the wax.

SUMMARY

There are three critical points near the transition point from solid to liquid wax. The first is the thaw point or the eutectic temperature of the wax, and this is approximated by the ring and plunger method. The second is the melt.point or the point at which all wax is actually liquid, best evaluated by the A.S.T.M. paraffin melting point (*1*) for all waxes. The third is the flow point or the point at which the viscosity is reduced to about 50 cs. best evaluated by the A.S.T.M. petrolatum melting point (*2*) method.

High melting, hard microcrystalline waxes are subject to viscosity hysteresis at the melting point, but other petroleum waxes are not.

ACKNOWLEDGMENT

The writer wishes to acknowledge the assistance of J. A. Graves, M. E. Bolton, and J. D. Anderson in accumulating the data presented in this paper.

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(6) Holde and Mueller, "Examination of Hydrocarbon Oils and Saponifiable Fats and Waxes," 2nd ed., New York, John Wiley & Sons, 1922.
(7) U. S. Army Ordnance, Method AXS 1015, 6-24-43.
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RECEIVED August 9, 1949.

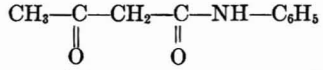
Correction

In the article on "Determination of Liquid-Vapor Equilibria" [Feller, Morris, and McDonald, H. J., ANAL. CHEM., 22, 338 (1950)], the last line on page 338 should read: "the sample is −0.1 mm. of mercury per millimeter." In Table II, page 340, the headings of the dew point and bubble point columns should be mm. Hg instead of ° C.

CRYSTALLOGRAPHIC DATA

31. Acetoacetanilide I (Acetoacetic Anilide)

Contributed by J. KRC, JR., AND W. C. McCRONE, Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.



Structural formula for acetoacetanilide

GOOD crystals are obtained from alcohol, water, dioxane, ethyl acetate, acetone, and benzene solutions (Figure 1). Figure 2 shows an orthographic projection of a typical crystal from solution. Four polymorphs have been observed from fusion (*1*); however, only the stable modification I was obtained from solutions with various solvents and varying conditions of crystallization.

A *B*

Figure 1. Acetoacetanilide

A. **Crystals of modification I from benzene (parallel Nicols)**
B. **Fusion preparation showing Modifications I, III, and IV (crossed Nicols)**

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.

Form and Habit. Hemimorphic tablets or rods elongated along *c*, lying on macropinacoid {100} and showing forms {010}, {001}, {021}, {041}, and {110}.

Axial Ratio. $a:b:c = 0.573:1:0.450$.

Interfacial Angles (Polar). $021 \wedge 0\bar{2}1 = 83° 55'$; $041 \wedge 0\bar{4}1 = 121° 50'$; $110 \wedge 100 = 29° 40'$.

Cleavage. {100} and slight {010}.

X-RAY DIFFRACTION DATA

Cell Dimensions. $a = 11.07$ A.; $b = 19.31$ A.; $c = 8.68$ A.

Formula Weights per Cell. 8.

Formula Weight. 177.20.

Density. 1.23 (pycnometer); 1.26 (x-ray).

Principal Lines

d	I/I_1	*d*	I/I_1
11.13	1.00	2.27	0.08
9.61	0.19	2.21	0.08
6.43	0.04	2.14	Very weak
5.54	0.28	2.10	0.08
5.26	0.18	2.06	Very weak
4.68	0.30	2.02	0.14
4.50	Very weak	1.966	0.17
4.36	0.37	1.903	Very weak
4.20	0.89	1.864	0.07
4.03	0.62	1.825	0.07
3.93	0.49	1.786	Very weak
3.79	0.13	1.752	Very weak
3.63	0.29	1.702	0.08
3.37	0.64	1.645	Very weak
3.22	0.16	1.607	0.03
3.09	0.09	1.580	Very weak
3.01	0.27	1.482	0.03
2.90	Very weak	1.451	Very weak
2.78	0.33	1.386	Very weak
2.67	0.11	1.356	Very weak
2.55	0.11	1.166	0.03
2.42	0.12	1.072	Very weak
2.32	0.13		

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). $\alpha = 1.556 \pm 0.002$; $\beta = 1.603 \pm 0.002$; $\gamma = 1.697 \pm 0.002$.

Optic Axial Angles (5893 A.; 25° C.). $2V = 74°$ (calcd.); $2E = 150°$ (calcd.).

Dispersion. $r > v$.

Optical Axial Plane. 010.

Sign of Double Refraction. Positive.

Acute Bisectrix. $\gamma = a$.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.618$. R(calcd.) = 49.5. R(obsd.) = 50.4.

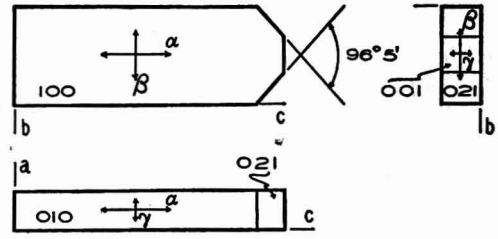


Figure 2. Orthographic Projection of Typical Crystal of Acetoacetanilide I from Benzene

FUSION DATA

Acetoacetanilide shows four polymorphic forms when crystallized from fusion. If fused completely and allowed to cool, any of these modifications may crystallize, either spontaneously or with application of pressure on the cover glass. Forms I (m.p. 83° C.) and IV grow rapidly as highly birefringent rods or plates, the latter being transformed almost immediately by I from many nuclei. This rapid transformation made it impossible to determine melting point of modification IV. Modification III (m.p. 61° C.) solidifies spontaneously from a supercooled melt as very low birefringent spherulites (first-order gray to yellow). Modification II (m.p. 72° C.) seldom crystallizes spontaneously. It can be observed readily, however, in the transformation III → II at lower temperatures, crystallizing into fine radiating crystals from many points of nucleation. Transformation III → I is rapid but not nearly so rapid as IV → I. Transformations IV → III, III → II, and II → I are relatively slow. Figure 1,*B*, shows two crystal fronts approaching each other. The leading white edges are IV and the dark portions immediately behind IV are I resulting from the transformation IV → I. The other white crystals are I crystallized spontaneously from the melt excepting the small white upper right section, which is modification III.

LITERATURE CITED

(1) Kofler, A., *Mikrochemie ver. Mikrochim. Acta*, 34, 15–24 (1948).

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago, Ill.

Correspondence

Determination of Carbon in Ferrous Alloys

SIR: In the report of the Atlantic City round-table discussion on the "Determination of Carbon in Ferrous Alloys" [ANAL. CHEM., 22, 488 (1950)] I note some errors in reporting my portion of the discussion.

This occurs on page 488, second column, and third paragraph.

The last part of the second sentence in this paragraph and following sentences should read: "so a study was made of the variation of carbon with depth and position of drilling. The carbon content of the top centers of the blocks were as much as 0.3% higher than the bottom centers. As a result, the present procedure is to cast a block in a metal mold, and drill the sample from the top through the center of the casting as far as the drill will go. This procedure gives the most uniform quick sample."

JOHN R. BOYD

James B. Clow & Sons
Coshocton, Ohio

Dichromate Reflux Method for Determination of Oxygen Consumed

SIR: It would appear that the authors of the above paper [Moore, Kroner, and Ruchhoft, ANAL. CHEM., 21, 953 (1949)] have overlooked the fact that silver is an efficient catalyst for the oxidation of acetic acid by dichromate–sulfuric acid mixtures [see, for example, Nicloux, *Compt. rend.*, **184**, 890 (1927); Cordebard and Michle, *Bull. soc. chim. France*, **43** (i), 97 (1928).

In a paper by the writer [Muers, *J. Soc. Chem. Ind.*, **55**, 71T (1936)] a method was described for estimating the strength (oxidizability) of dairy effluents containing lactic and acetic acids and ethyl alcohol. In the presence of 0.25% silver sulfate all three substances were quantitatively oxidized under conditions otherwise similar to those used by Moore *et al.*

The experimental work, which was only summarized in the published paper, did not cover all the pure substances examined by Moore *et al.*, but it seems probable that the silver catalyst would have improved their results in most cases where there was a large deviation from the theoretical oxygen consumption.

M. M. MUERS

Central Laboratory
United Dairies Ltd.
London W. 12, England

SIR: We wish to thank Muers for calling our attention to this rapid method for estimating the strength of a solution containing organic matter. Unfortunately, Muers' procedure was published as a part of a study on the biological purification of whey solutions. The procedure for oxygen consumed used by Muers in this study was not stressed or given in detail; consequently, it was not cross-indexed in *Chemical Abstracts*. Therefore, in our bibliography search on procedures for oxygen consumed this particular method was not found. This center has not followed directly studies on the purification of whey wastes and, therefore, we were not familiar with Muers' work. An examination of Muers' method indicates that it followed the same general procedure as that used by Ingols in this country. The procedure used by this laboratory is somewhat different in detail.

This center is now engaged in a comprehensive comparative study of five procedures for evaluating the oxygen-consumed values of sewages and industrial wastes. In these studies between 50 and 100 replicates of various wastes are being examined by each procedure and statistical analyses are being made on the results, so that the most desirable method can be determined.

As soon as Muers' suggestion was received, Moore and Ludzack of this laboratory undertook an evaluation of the effectiveness of silver sulfate as a catalyst. To date completed studies, using 100 replicates of a large number of wastes, have shown that the procedure suggested by Moore *et al.* [ANAL. CHEM., 21, 953 (1949)] had advantages over all others. Consequently, the effectiveness of silver was studied by introducing the quantity of silver suggested by Muers in our dichromate reflux method. Ten replicate portions of 18 organic chemicals have been examined to date with this procedure both with and without silver. This preliminary study has shown that the silver sulfate is very effective in catalyzing the more complete oxidation of most of these compounds. The oxidation of compounds, such as castile soap, caproic acid, glutamic acid, acetic acid, lactic acid, butyric acid, *o*-cresol, furoic acid, and ethyl alcohol, is raised to values between 80 and 98% by the addition of the silver. Chlorobenzene and spirits of turpentine are oxidized to 41.4 to 46.8% of theoretical by the addition of the silver salt. A few compounds, such as benzene, toluene, and pyridine, are apparently not affected by the addition of the silver and show very little, if any, increase in oxidation.

Studies on the dichromate method for oxygen consumed with and without the addition of silver will be continued and a report on this work will be prepared in the future. Cooperative work with the American Society for Testing Materials subcommittee on water-borne industrial wastes and the Committee on Standard Methods for the Analysis of Water and Sewage on this method is being carried on. As a representative of both these committees, I again thank you for bringing Muers' paper to our attention.

C. C. RUCHHOFT

Public Health Service
Federal Security Agency
Cincinnati, Ohio

Book Reviews

Vitamin Methods. *Paul György*, editor. Vol. I. x + 571 pages. Academic Press, Inc., 125 East 23rd St., New York, N. Y., 1950. Price, $10.

This book is the first of two volumes which together will constitute a comprehensive treatise on vitamin methods. In the development of the subject matter, the presentation has been built around the various techniques employed for assays (physical, chemical, microbiological, and animal assays) rather than the various vitamins.

In the preparation of Volume I, György has had the assistance of the following collaborators: Eric T. Stiller, Physical Methods; Saul H. Rubin, Chemical Methods; Otto A. Bessey, Microchemical Methods; Esmond E. Snell, Microbiological Methods; Erich Hirschberg, Use of Optical Instruments.

Where several alternative methods are available for a given physical, chemical, or biological assay of a vitamin, these are described in sufficient detail to obviate the necessity for consulting the original sources. Where possible, the most reliable and widely used procedure is indicated. One of the significant features of this volume is the concise and direct approach employed in presenting the various steps involved in the assays.

Preceding the actual details of the assay for each vitamin, a section is devoted to the historical development of the analytical procedure. Pertinent explanatory material relating to the application and interpretation of the technique is usually incorporated in this section, rendering such valuable information available without encumbering the actual laboratory directions. Definite statements on accuracy, precision, and limitations are usually presented if available.

Certain sections have not included as much of the more recent literature as might have been possible. Thus, the section on physical methods, with 346 references, discusses only three papers published subsequent to 1946. The book will be of value to workers in various fields concerned with nutritional problems.

E. M. BICKOF

The Chemistry of Industrial Toxicology. *Hervey B. Elkins.* ix + 406 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, N. Y., 1950. Price, $5.50.

This useful book, by a well recognized industrial hygienist, will be of greater value to the individual with limited knowledge of the problems of industrial evironmental control than to the experienced hygienist.

In the largest subject division, more than 300 elements, inorganic compounds, and organic compounds are briefly discussed with respect to their industrial hygiene significance, and many of them are qualitatively related, as to hazard, to the more common industrial operations and processes. In this, the author has incorporated much useful experience of the Division of Occupational Hygiene of the Massachusetts Department of Labor and Industries.

In developing the subject of "maximum allowable concentrations," and including a further modified list of values, the author's statement "values based on inadequate data are enclosed in parentheses" may imply to the less experienced in this field a reliability for the values not so qualified which many do not have. The sections on air-sampling devices and analytical methods and procedures are well organized. The abridged experience with respect to such items as the use of activated silica gel and carbon and of absorption in nonreacting liquids will be useful to the newcomer to this field.

For the majority of the approximately 100 substances listed, only one analytical method is given. These are usually the generally accepted procedures and are well selected as to adaptability by a good analytical laboratory. In general, the descriptions are sufficiently complete so that a reasonably good analyst could carry out the procedure without having to refer to the original literature.

JAMES H. STERNER

Précis d'Analyse Qualitative. *Robert Flatt.* 237 pages. Libraire de l'Université F. Rouge et Cie., SA, Lausanne, Switzerland, 1949. Price, $6.50 (28 Swiss francs).

The first part of this excellently organized and well written book summarizes in concise, easily understandable form those basic principles of physical chemistry which a student must grasp and apply if qualitative analysis is to be more to him than cookbook procedure. The basic techniques of laboratory experimentation and observation are separately summarized and taught by a special group of experiments. A systematic concise description of the reactions of the commonly encountered ions provides the basis for presenting the scheme of analysis in the form of a series of tables. Methods for fluxing insoluble substances, short cuts for rapid orientation, and possible sources of interference and difficulty are given full, yet concise consideration. The book is so well balanced and well written throughout that it is impossible to select any one section as better than the others.

J. W. PERRY

Electron Microscope—Technique and Applications. *Ralph W. G. Wyckoff.* vii + 248 pages. Interscience Publishers, Inc., 215 Fourth Ave., New York 3, N. Y., 1949. Price, $5.

The subtitle is very appropriate, for less than 20% of the book is devoted to microscopes, their construction and adjustment; the remaining 80% covers the preparation of specimens for examination, typical electron micrographs, and an interpretation of their significance. The book is primarily an account of the author's experiences; about 175 micrographs out of the 202 illustrations were made in his laboratories. The reproductions are of high quality, their purpose is explained, and the author's interpretation is clearly stated. A complete bibliography by topics is given at the end of the chapters.

Metal shadowing as a technique is given a full chapter and is used almost universally on all micrographs. Surface replicas are treated with emphasis on crystals and body tissue such as teeth rather than the metallurgical field. Chapters on particle suspensions, viruses, and macromolecules reflect the extensive experiences of the author in microbiology. A discussion of the structure of macromolecular solids introduces a visual or mechanistic approach to colloid chemistry and provides a common meeting ground between the chemist and the biologist.

The author concedes that electron microscopy is in a high state of flux and that the book describes the present status, to point out and arouse interest in the possibilities of this new instrument. Newcomers to the field of electromicroscopy will find the book a pleasant but thorough introduction to the subject. Those engaged in the field will value it for placing much experience and many quick references under one cover.

DONALD L. KATZ

Modern Industrial Spectrography

A two-week intensive course in modern industrial spectrography is to be given by Boston College, Chestnut Hill, Boston, Mass., from July 24 to August 4, 1950, particularly designed for chemists and physicists from industries in the process of installing spectrographic equipment. Applications should be sent to James J. Devlin, Physics Department, Boston College, Chestnut Hill 67, Mass.

The Analyst's Calendar

International Congress on Analytical Chemistry

Plans for holding an International Congress on Analytical Chemistry in Britain in 1952 are progressing. The Council of the International Union of Pure and Applied Chemistry has granted its patronage. The proposal was thought to be in accord with one of the major decisions taken at the September meeting of the International Union, when six autonomous sections were formed, one of them concerned with analytical chemistry. A meeting of the Section on Analytical Chemistry is expected to be arranged to coincide with the 1952 International Congress on Analytical Chemistry.

The Executive Committee, under the chairmanship of the president of the Society of Public Analysts and Other Analytical Chemists, G. Taylor, has under consideration the location of the congress, publication of papers, and the scope of subjects to be discussed. Sir Wallace Akers has been made chairman of the Finance Committee and Sir Robert Robinson, president of the Royal Society, is acting as chairman of the General Committee. The honorary secretary of the congress is R. C. Chirnside, Research Laboratories, General Electric Co., Ltd., Wembley, England.

International Microchemical Congress. Graz, Austria, July 2 to 6

Electron Microscope Society of America. Hotel Statler, Detroit, Mich., September 14 to 16. Eighth annual meeting

Instrument Conference and Exhibit. Instrument Society of America, Buffalo, N. Y., September 18 to 22

Fourth Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 29 to February 1, 1951

AIDS FOR THE ANALYST . . . . .

Simple Automatic Pipet. William Nye, Department of Chemistry, Stanford University, Calif.

An increasing number of biochemical methods employ automatic pipets. Although two types are now on the market, the pipet described can be made from readily available materials, exposes only glass and mercury to the liquid pipetted, and has very few moving parts, and the electrical portion can serve a number of glass pipets by transferring the solenoid coil. The flow of liquid is controlled by a double glass valve with an iron core. When the current flows through the solenoid coil, the lower valve opens and the upper valve closes. When no current flows, the top valve is open and the lower is closed. The fluid flowing in the upper valve displaces mercury until the current shuts off, then flows out through the lower valve.

Figure 1 illustrates the principal features. The magnetic valve assembly was made by sealing 3-mm. capillary tubing onto each end of a 12-cm. length of 12-mm. borosilicate glass tubing, and a side arm of 3-mm. capillary tubing (used to give added strength for support of the mercury-filled U-tube) was sealed on at an angle, which allows air to be displaced from the U-tube during filling. Then the 12-mm. tubing is cut just above the side arm. The magnetic valve is made by sealing a 6-mm. rod in one end of a length of 1/4-inch iron rod, constricting the tubing just above the iron, and sealing a 6-mm. borosilicate glass rod into the other end of the tubing. The rod at either end is drawn to a point, so that the assembly will move freely for 2 to 5 mm. along the length of the cylinder. Each end is seated to a water-tight fit in its end of the cylinder by grinding with fine emery dust; very little grinding is necessary, if the tips of the rods are radially symmetrical. The valve is then placed inside the cylinder and the cylinder is sealed. It is important that this assembly be kept straight and that a 12-mm. tubing fit into the 1/2-inch brass tubing core of the solenoid coil.

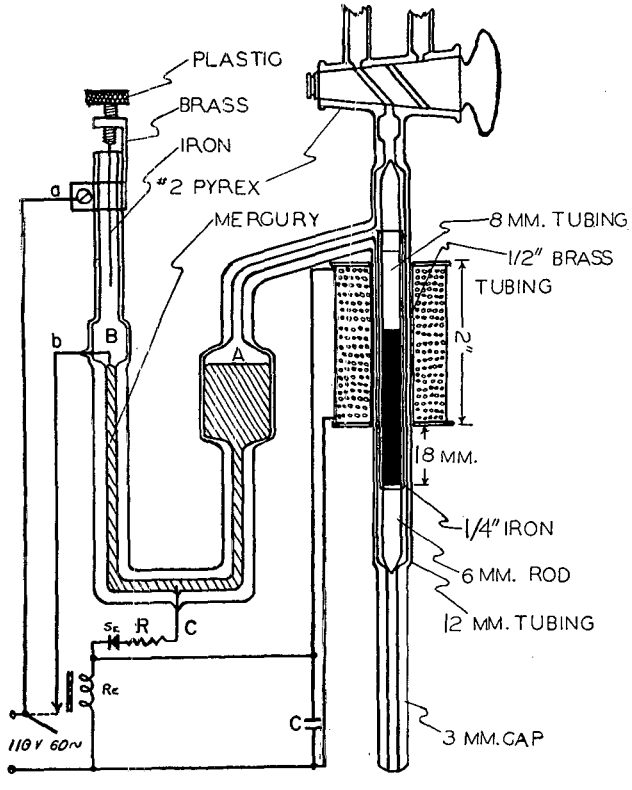


Figure 1. Automatic Pipet

Re. Relay 1000 ohms or more, S.P.S.T. normally open. *Se.* Selenium rectifier, Seletron 5M1. *R.* 25-ohm 2-watt resistor. *C* 20-mfd. 300-volt electrolytic condenser

The diameter of bulb *A* should be relatively large, in order to reduce the pressure necessary to operate the pipet, and the top of the bulb should be located approximately on the same level as the center contact, *b*. The volume of *B* is about 1 ml. less than the volume to be pipetted and *B* may be eliminated for small volumes if the proper size of glass tubing is chosen. The volume delivered by the pipet is determined by the volume between *a* and *b*, which are pieces of tungsten wire sealed into tubing.

The solenoid coil consists of 0.5 pound of No. 34 enameled copper wire wound on a spool made with a 1/2-inch brass tube 2 inches long and 1/32 inch in wall thickness. Such a coil supplies force to keep the upper valve closed when the rectified current from a 110-volt alternating current course is put through it.

All exposed wiring should be covered by tape, rubber tubing, or some other suitable insulation.

Filling and Operation. The U-tube is filled with mercury to a level just below *b*. The fluid to be pipetted may be placed in a reservoir at sufficient height to raise mercury in the outer side of the U-tube slightly above *a*, or this pressure may be attained by using a closed reservoir under proper gas or air pressure. The pipet is filled with fluid by alternately turning the two-way stopcock to the reservoir and to the air until all air in the pipet is displaced by fluid. If the stopcock is left open to the reservoir and the electrical circuit is complete, the pipet will repeatedly deliver fluid equal to the volume between *a* and *b*.

Accuracy. A 1-ml. pipet delivered the desired volume at 4-second intervals with a maximum difference of 0.004 ml. (0.4%). A 5-ml. pipet delivered at 4-second intervals with a maximum difference of 0.030 ml. (0.6%). The maximum difference may be decreased by decreasing the rate of filling and/or emptying.

This work was done during a research assistantship on a project supported by the United States Public Health Service.

Dispersion of Fine Particles for Electron Microscopy. Vincent Salines, Interchemical Corporation, New York, N. Y.

In the electron microscopy of pigment particles, it is often difficult to make very fine dispersions. The author has found the following method generally satisfactory.

The pigment powder—carbon black, phthalocyanine, benzidine yellow, etc.—is dried in an oven at low heat, usually 40° to 80° C., to remove as much moisture as possible without changing its particle characteristics.

With the tip of a stiff spatula, 0.10 to 0.25 gram of the pigment is transferred to a white Carrara glass slab (ground with 320-mesh abrasive to impart "tooth"). On this small mound of pigment is dropped 1 to 2 ml. of a solution containing approximately 10% of nitrocellulose in Cellosolve acetate (nitrocellulose in stick form offered by Mallinckrodt Chemical Company under the name Parlodion has been found satisfactory). By means of the spatula, the mixture of pigment and solution is rubbed out; as the solvent is lost and dryness approaches, the rubbing is stepped up in vigor and speed, in order to obtain the maximum shearing effect and dispersion of the pigment in the binder.

When the mixture appears dry, a few drops of distilled octyl acetate (2-ethylhexyl acetate) are added and the mixture is covered with a Petri dish. In a short time, the mass will be softened by the solvent action of the octyl acetate. A few more drops of solvent are added and the mixture is again worked vigorously with the spatula until it appears to be fully homogeneous. One small drop of it is then allowed to fall on the swept surface of distilled water in a paraffin-coated dish. It will spread out into a very thin film. Evaporation of the octyl acetate may be hastened by use of an infrared lamp. The period of evaporation, using the infrared lamp, is between 2 and 3 minutes. Lectromesh (200-mesh) disks (C. O. Jelliff Manufacturing Company) are dropped shiny side down onto the film and picked up in the usual manner to be dried before insertion into the electron microscope.

All solvents must be freshly distilled, because water and other contaminants cause the film to be fragile and lacy. The Parlodion solution must contain no plasticizer, as this produces films which creep in the electron beam.