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INDUSTRIAL AND ENGINEERING CHEMISTRY
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Flame Photometry

A Rapid Analytical Procedure

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A new instrument has been developed to make possible the rapid quantitative determination of the alkali metals (primarily sodium and potassium) in aqueous solution. The principle of operation of the instrument is based upon the quantitative measurement of the characteristic light emitted when a solution of the metal is atomized as a mist into a gas flame. Details of construction and operation are given. An average solution may be analyzed for both the sodium and potassium contents in a few minutes' time with an average accuracy of ±3% of the amounts of these elements which are present. Several applications of the method are discussed, together with the analytical procedures employed.

THE urgent need for extremely rapid and accurate sodium determinations on large numbers of samples led to the development of the flame photometer. With this instrument as many as 150 sodium determinations on water samples low in sodium have been completed in a single hour's time with an average accuracy of about ±3% of the amount of the sodium present. The flame photometer is simple in construction, its operation requires no excessive training on the part of the analyst, and the samples used require a minimum of preparation. Although originally constructed for performing sodium determinations, its usefulness has been extended to include other alkali metals and various alkaline earths.

A survey of previously used chemical methods, including the zinc uranyl acetate method of Barber and Kolthoff (*1*), revealed that satisfactory quantitative sodium determinations require a considerable amount of sample preparation and are rather time-consuming. This is particularly true in the cases of samples in which only traces of sodium are present, or which contain other cations in appreciable quantities. Accordingly, a physical approach to this analytical problem was undertaken in the hope that a suitable method could be found which would meet the requirements of analytical speed and accuracy, would necessitate a minimum of sample handling and preparation, and at the same time would not require the use of expensive or complicated equipment. Although many well-known physical methods were tried and tested, only flame photometry was found to satisfy all the above requirements.

Possibly the most widely known spectroscopic phenomenon is the fact that sodium when introduced into a flame emits a characteristic yellow light, its intensity being a function of the amount of sodium present in the flame. This emission by atoms of characteristic radiation when thus excited by high temperatures or by electrical means forms the basis of the analytically important subject of emission spectroscopy. A review of the spectrochemical procedures employed for the determination of sodium indicated that the Lundegårdh method (*3*, *6–10*) has been successfully applied to the analysis of many metallic elements including the alkali metals. In this method, which has recently been modified and called the air-acetylene flame method (*2*), the sample to be analyzed is put into aqueous solution and sprayed under controlled conditions into an acetylene flame. This is usually accomplished by means of a specially constructed atomizer and burner in which the air and acetylene are carefully regulated to produce constant burning conditions. The light from the Lundegårdh flame enters the slit of a spectrograph and spectrograms are prepared, which in turn may be photometered in order to obtain the intensities of the characteristic spectral lines recorded After carefully calibrating with solutions of known composition and concentration, it is readily possible to correlate the intensity of a given spectral line, such as the sodium line, of the unknown sample with the amount of that particular element present. The use of the high-temperature acetylene flame causes a great many of the metals contained in the sample to emit characteristic radiations. This fact has led most workers to the use of a spectrograph to isolate and measure the intensities of these spectral lines. Although accurate and versatile, this method requires a very considerable amount of manipulation and equipment.

Jansen, Heyes, and Richter (*5*), by making use of a monochromator to isolate the desired region of the spectrum and a photocell and electrometer to measure the intensity of the lines, successfully determined the alkali metals and alkaline earths in solutions sprayed into a flame, while Heyes (*4*) later succeeded in applying the method to other metals.

A somewhat simpler technique for the determination of potassium was developed by Schuhknecht (*12*). The apparatus consists of a Lundegårdh acetylene flame source placed before a box containing a phototube and suitable filters for isolating the red potassium wave lengths. The method of operation is similar to that described below. Apparently the Schuhknecht method has been applied with considerable success in the analysis of samples of soils, fertilizers, and plant materials in Germany. Two instruments based on this principle have been manufactured by German companies (Siemens and Zeiss) and descriptions of both can be found in an article by Schmitt and Breitweiser (*11*).

Consideration of the flame as a source led to the development of a lower temperature method described below, which is called flame photometry.

[1] Present address, Fisher Scientific Company, Pittsburgh, Pa.

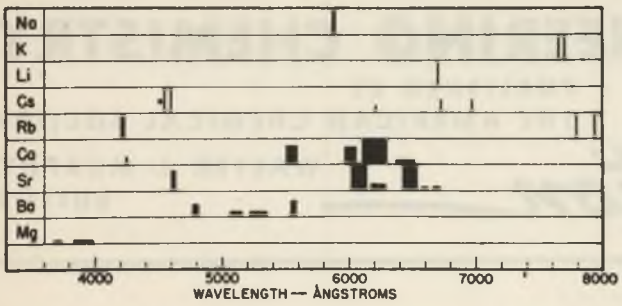

Figure 1. Characteristic Flame Spectra Produced in Illuminating Gas Flame

FLAME PHOTOMETRY

A very simple instrument has been developed wherein a solution to be analyzed is atomized into the air intake of a flame under controlled conditions and the emitted light characteristic of the element in question is isolated and accurately photometered. If the temperature of the flame is not too high, only a few of the metals which may be present in the sample will be caused by thermal excitation to emit characteristic radiation. In particular, if one uses as a fuel for the flame ordinary illuminating gas or cylinder gas (Pyrofax), only the atoms of the alkali metal and the alkaline earth constituents of the sample will emit light in appreciable amounts. This is particularly important, since it is obvious that the fewer the components of the sample which are excited, the simpler the task of isolating for photometric purposes the light characteristic of any one component.

Figure 1 shows the most prominent wave lengths emitted by various metals when excited by a flame of the type described above. It may be readily demonstrated that any one of these characteristic radiations, if isolated by passage through proper filters, will, upon striking a photosensitive element, give rise to an electrical impulse which is a function of the quantity of the respective metal present in the sample. The above is true provided the burning conditions of the flame and the rate of atomization can be kept constant. The flame photometer is an instrument wherein the above requirements for analysis may be met.

LABORATORY MODEL FLAME PHOTOMETER

A recent model flame photometer for routine laboratory use is shown in Figure 2. The schematic drawing of this instrument, Figure 3, indicates the arrangement of its various parts.

In successful flame photometry, the chief prerequisite is the establishment of sufficiently stable flame conditions, so that solutions of various concentrations may be successively brought to excitation in the flame without such conditions changing sufficiently to influence the quantitative light output being measured.

R and R^2 are pressure regulators controlled by knobs K and K^2, for the atomizer air and the burner gas, respectively, the pressures used being indicated by G and G^2. The operator may readily establish identical conditions each time the photometer is placed in operation. The flow of air through capillary E past the tip of the sample tube, F, atomizes the sample solution which is introduced through the funnel, W. The larger droplets of the solution condense on the inner walls of the glass atomizer bulb, A, and run off to the drain, D, while a fine mist of the sample is carried by the air stream to the mixing chamber, C, at the base of the Fisher burner where it is thoroughly mixed with the gas entering the burner. Natural draft created by the burner prevents the escape of any of the mist through the loosely fitting collar of C; indeed, very considerable air in excess of that supplied through the atomizer is drawn in around this collar to support the combustion of the flame.

Whereas the atomizer tips may be made of glass as indicated in Figure 3, a preferred atomizer may readily be made using stainless steel hypodermic needles (see Figure 4). This type of atomizing unit is introduced into a chamber similar to the one in Figure 3, but with a large opening in the top of the bulb into which the unit fits by means of a rubber stopper.

Burner B, Figure 3, is supplied with regulated gas, its flow being controlled by the needle valve, N. In burning certain types of bottled gases, this needle valve has been replaced by a fixed orifice.

Light from the flame passes to the optical system of the photometer through a window, H, in the chimney, Q, so dimensioned as to exclude all light from the grid of the burner and from the unsteady edges and top of the flame. The light then passes horizontally through lens L^2, is reflected downwards by the 45° mirror, M, through lens L and through the filter system housed in container V to the coolest part of the instrument, where it falls upon the surface of the barrier layer photocell, P. Wires, not shown, connect the photocell through the toggle switch, T, to coarse and fine controlling rheostats and finally to the galvanometer jacks, J. The rheostats, S and S^2, enable the operator to control the sensitivity of the instrument by shunting the galvanometer, while retaining the desired damping resistance.

While any galvanometer may be used which has sufficient sensitivity, and a critical damping resistance compatible with the resistance of the photocell, the authors prefer one of the indicating box type as shown in Figure 2.

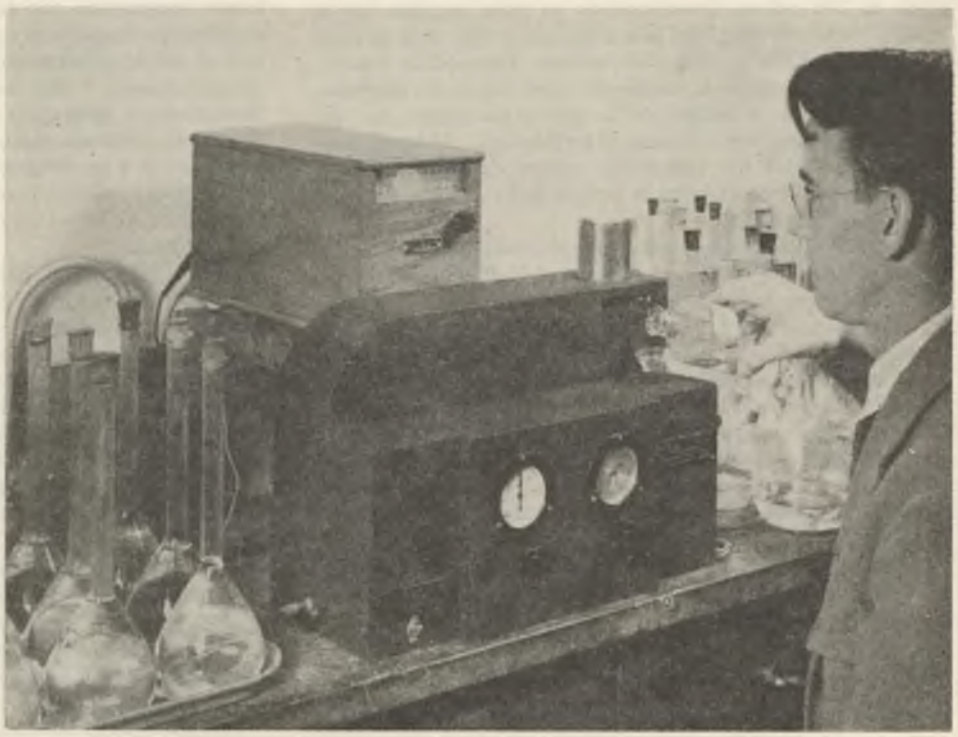

Figure 2. Laboratory Model Flame Photometer

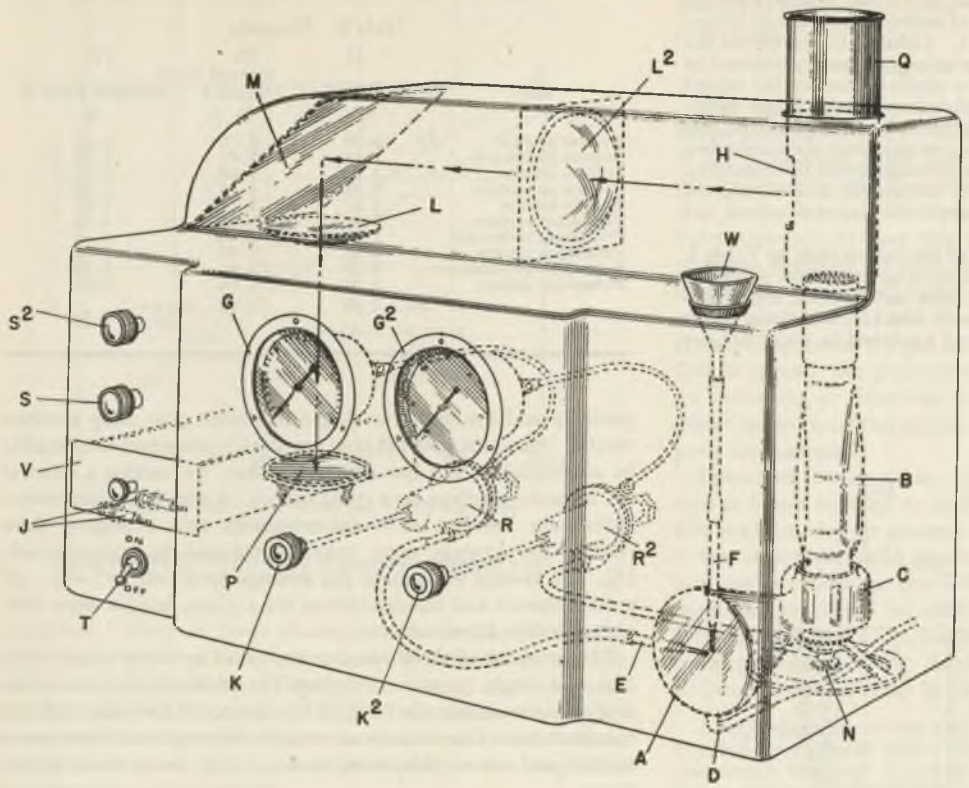

Figure 3. Diagram of Flame Photometer

ANALYSIS FOR SODIUM OR POTASSIUM

In using the flame photometer the procedure is extremely simple. A set of concentrated stock solutions containing, say 1000 p.p.m. of sodium and potassium, respectively, are prepared and stored in glass-stoppered No-Sol-Vit bottles. These stock solutions may be preserved for several months, and from them dilutions to any desired concentration may be made very readily. Once satisfactory conditions have been established for the gas and the air pressures, the diluted standards may be used to calibrate the instrument for a variety of ranges for each element. As long as the flame and atomizer conditions are kept constant, it is usually only necessary to check the upper and lower ends of the proper range calibration curve when placing the instrument in operation.

The procedure for calibrating the instrument for a given metal is as follows:

With the flame burning properly and the correct filters in place, distilled metal-free water is introduced into the atomizer, and the galvanometer is set to read zero.

After the zero reading is set, the sensitivity can be adjusted by the introduction of the standard solution selected to be the upper end of the desired concentration range. To do this the control rheostats are adjusted so that the galvanometer reads 100. Several standards of lower concentration are introduced in turn and the respective readings noted. These readings, plotted as ordinates versus the concentrations as abscissas, result in a satisfactory calibration curve. Once the proper calibration curve has been established, it is unnecessary to repeat this entire procedure. It is only necessary, at the beginning of each set of readings, to adjust the instrument so that demineralized water reads 0 and the upper standard chosen reads full scale or 100. For exact work these two readings are checked after the analysis of each three or four unknowns.

For practical reasons it is usually desirable to dilute most unknowns to 100 p.p.m., or less, of the metal; the reason for this is evident from a study of Figure 5, which shows four different calibration curves for a flame photometer of the type shown in Figure 2.

Curve 1, in which 10 p.p.m. are set to read 100 on the galvanometer, is so nearly linear that results may be interpolated directly from the meter readings. Curve 2, 100 p.p.m. full scale, is not so nearly linear, and accordingly the use of a calibration curve is recommended. Curves 3 and 4, with 1000 and 10,000 p.p.m. at full scale, are very appreciably curved and become very low in slope toward the upper end of the range. Probably self-absorption—i.e., absorption of the sodium light by the cooler atoms around the periphery of the flame—plays a role in this dropping off of sensitivity. In general, it will be most convenient to dilute the average sample to a range below 100 p.p.m., so curve 2 may be used. By such dilutions, the dangers of excessive contamination and corrosion of the burner are avoided and smaller samples of the original solutions will be required.

When using such highly diluted samples, care must be exercised to avoid contamination of the sample. Such solutions, for example, cannot be kept safely for any length of time in ordinary glass containers, because of the likelihood of contamination from the glass. In transferring these highly diluted solutions, care must be taken not to touch the inside of any of the glassware, stoppers, etc. An interesting demonstration may be made by filling the atomizer funnel with metal-free water and then dipping a finger tip into this water for about one second. A reading of 10 to 20 p.p.m. of sodium is usually obtained.

The sensitivity of the instrument shown in Figure 2 is such that 10 p.p.m. of sodium (or 50 p.p.m. of potassium) may easily be set to read full scale on the galvanometer (100 divisions). The normal rate of atomization of a sample into the instrument is in the order of 5 to 10 ml. per minute. It is thus possible to determine the metal content of a 2- or 3-ml. sample. In general, however, it is preferable to have on hand a sample of approximately 10 ml. for assurance in determining the true rest point of the galvanometer.

ACCURACY OF THE METHOD

In order to determine the average accuracy of the flame photometer, two series of fifty solutions each of sodium and of potassium, respectively, were prepared and submitted to a second staff member for analysis as "unknowns". In preparing these standard unknowns, pure sodium and potassium carbonates were obtained, weighed accurately into volumetric flasks, and made slightly acid by the addition of various acids, thus forming samples of nitrates, phosphates, sulfates, acetates, and carbonates in which the amounts of the various cations were accurately known. Since the purpose of this experiment was to determine the average accuracy of the method under conditions duplicating those of actual daily analyses, the same procedure was followed in each case. Each sample was atomized once in the instrument and one galvanometer reading taken, the operator then proceeding to the next sample. After completing each series of unknowns, the above measurements were repeated. As standard solutions for calibration in these experiments, pure sodium and potassium chloride solutions were used, made up from analytical grade reagents.

Table I is typical of the manner in which the results were recorded and averaged. Ten solutions of sodium carbonate of known concentrations are listed in column I. Column II represents the analysis obtained the first time the solutions were atomized in the instrument, and column IV gives similar data for the repeat analysis. Columns III and V show the errors for the single determinations of analyses 1 and 2, in terms of per cent of the element present. Column VI shows the average error obtained when analyses 1 and 2 were averaged. The average errors (disregarding sign) for the first and second set of ten single determinations, as well as the average error of the duplicate determinations, are shown at the bottom of the table.

Table II represents the summary of ten tables such as Table I. The ten salts used to make the analytical solutions are shown in column I. Average errors for the first and second set of ten single analyses are shown in columns II and III, and the average errors for each group of ten solutions analyzed in duplicate are shown in column IV.

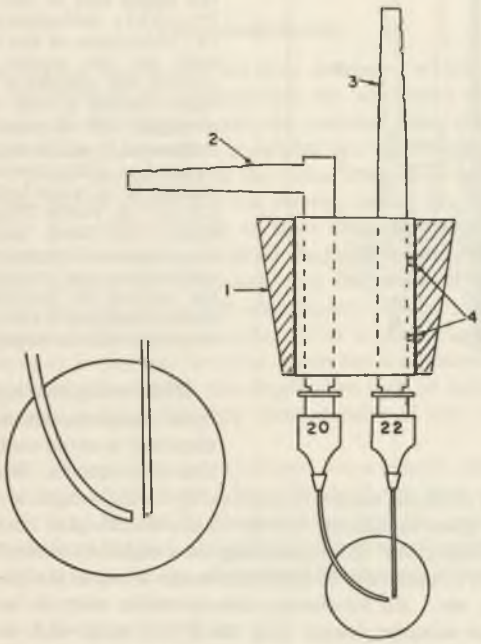

Figure 4. Metal Atomizer

Stainless steel hypodermic needles, 20- and 22-gage, used as tips

1. Rubber stopper
2. Air feed
3. Liquid feed
4. Adjusting screws

As may be seen, the average error made in 200 single determinations is ±3.0% of the amount of metal present, whereas that for samples determined in duplicate is ±2.8%. Such results compare favorably with those obtained by standard spectrographic procedures and also by chemical procedures where dilute solutions of sodium or potassium are being analyzed.

In certain test cases individual samples have been analyzed repeatedly and the results averaged to obtain even more accurate results. Since the method is so rapid, this procedure may readily be accomplished in a few minutes. Thus, by making a series of ten separate readings on a given sample, checking the instrument calibration between each determination and averaging these results, the average error may be reduced to approximately 1%. In twenty such cases the average error was ±1.4%. All measurements and manipulations for a given sample were completed within 15 minutes.

The precision of the method as expressed by the average deviation of a single measurement from the arithmetical mean of ten single determinations is 2.8% of the mean. This value was also obtained from the analysis of twenty different solutions, ten of sodium and ten of potassium, each solution being analyzed ten times.

Table I. Sodium Carbonate

Solution	I Sodium Added	II Analysis 1	III Error	IV Analysis 2	V Error	VI Average Error
	P.p.m.	*P.p.m.*	%	*P.p.m.*	%	%
1	8.73	8.3	−4.93	8.7	−0.34	−2.63
2	13.1	12.9	−1.53	13.3	+1.53	−0.00
3	21.8	21.2	−2.75	21.5	−1.38	−2.07
4	26.2	26.8	+2.29	26.8	+2.29	+2.29
5	34.9	34.2	−2.00	35.1	+0.57	−0.72
6	39.3	40.4	+2.80	40.4	+2.80	+2.80
7	48.0	47.7	−0.63	48.8	+1.67	+0.52
8	56.8	57.8	+1.76	57.5	+1.41	+1.58
9	61.0	60.2	−1.31	63.0	+3.28	+0.94
10	78.6	80.0	+1.78	80.0	+1.78	+1.78
		Average error	2.18		2.01	1.53

Table II. Summary

I Salt	II Average Error Analysis 1	III Average Error Analysis 2	IV Average Error Analyses 1 and 2
	%	%	%
Sodium acetate	4.35	3.76	4.07
Sodium carbonate	2.18	2.01	1.53
Sodium nitrate	4.38	5.83	5.11
Sodium phosphate	2.34	1.89	1.92
Sodium sulfate	1.61	1.77	1.47
Potassium acetate	3.85	4.01	3.65
Potassium carbonate	2.42	2.15	1.44
Potassium nitrate	3.41	2.69	3.05
Potassium phosphate	3.17	3.95	3.55
Potassium sulfate	2.52	1.74	2.01
Av.	3.02	2.98	Av. duplicate 2.78

ANALYSIS FOR CALCIUM

It has long been established that the flame spectra of a number of elements are sufficiently unique to enable one to use them for analytical purposes.

The extension, however, of the above-described method to the analysis for metals other than sodium and potassium, while theoretically possible, is fraught with certain experimental difficulties. In general, analyses by flame spectra have been performed either as qualitative flame tests or as quantitative analyses in which some type of spectrometer was employed. In the case of the former, the human eye alone is used as the detector and since the eye does not resolve mixed colors into their component wave lengths, a wide range of flame shades must be

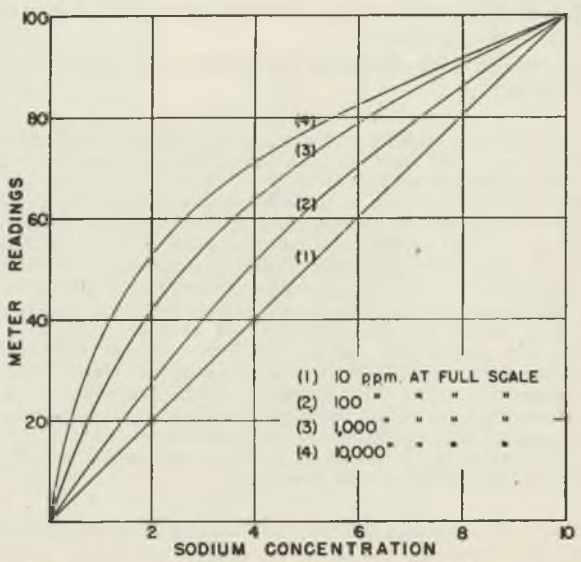

Figure 5. Typical Calibration Curves for Flame Photometer

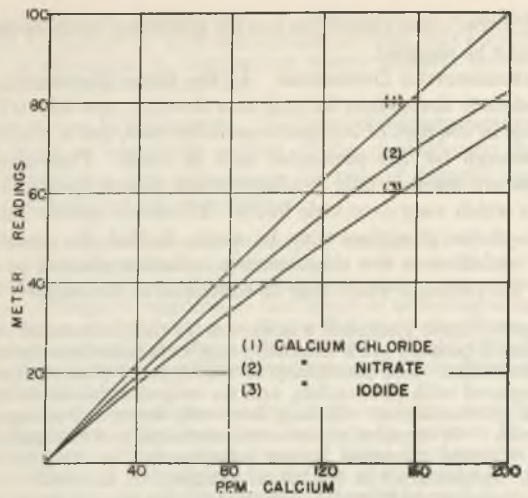

Figure 6. Calibration Curves for Three Calcium Compounds

Calcium chloride (200 p.p.m.) used to standardize instrument at full scale

recognized. Many of these shades can be correlated with the simultaneous presence of two or more elements in the sample introduced into the flame. Through the use of a spectrometer of sufficiently high dispersion, the light from flames can readily be resolved into characteristic lines and/or band systems which, when photometered, yield quantitative information regarding the composition of the sample.

In the design of the present flame photometer only a simple filter system has been provided; accordingly, the use of this instrument is limited to those samples containing elements whose flame spectra can be isolated by available filters. In many cases, however, examination of the flame with a small hand spectroscope provides additional information of a qualitative nature.

A considerable amount of work has been done on the application of this method to the determination of calcium. Already the flame photometer has performed well in the analysis of this element in many cases where sodium did not appear in high concentrations. For example, in samples in which only calcium and magnesium are present, the calcium content may be determined readily, as shown in Table III. Unfortunately, in the case of one of the most important clinical analyses—namely, the determination of blood and urine calciums—the situation is not entirely favorable. In such samples, sodium is approximately 25 times as prevalent as calcium, and with the best filter system so far found a correction of some 30 to 40% of the calcium reading has been necessary to account for the fraction of the sodium light passing the filters. It will probably be impossible to eliminate the necessity for such a correction by means of simple filters alone, for the sodium flame does actually emit some light in the spectral region of the calcium bands.

Another difficulty encountered in performing calcium analyses arises from the very origin of the calcium spectrum. In contrast to the alkali metals, which emit line spectra that are relatively easy to isolate by filters, calcium emission is largely a band system. Since this band system originates from the thermal excitation of calcium-containing molecules (probably the oxide or chloride), it might be expected that the anion associated with the calcium would exert some influence upon the intensity and character of the emission. Experimentally, this is the case, as may be seen from the calcium calibration curves of Figure 6. From these curves it is clear that the standards used in the flame photometry of calcium must be prepared from the same compound or compounds in the same ratio as that contained in the unknowns being analyzed. Otherwise, chemical pretreatment of all unknowns is necessary in order to convert all of the calcium to the same form.

CONSTRUCTION OF THE FLAME PHOTOMETER

The previous discussion has been limited to the particular flame photometer shown in Figure 2. Obviously the principle of the instrument lends itself to a wide variety of designs, depending very largely upon the specific use to which the photometer is to be put. As a matter of fact, several radically different instruments have been in use for some time. The simplest of these, designed for field work, is portable and makes use of bottled gas and a small motor-driven air compressor.

The photometer itself consists essentially of six parts—the pressure regulators for gas and air, the atomizer, the burner, the optical system, the photosensitive detector, and the instrument for indicating or recording the output of the detector. In the following sections the principal requirements for each of these parts are discussed.

PRESSURE REGULATORS. In most laboratories the gas pressure is found to vary to such an extent that adequate regulation is a necessity for accurate operation of the flame photometer. A few companies build pressure regulators which suitably regulate gas pressures at the low pressures generally prevalent in municipal supplies. In certain localities, however, the gas pressure will be found to be inadequate to provide a flame of proper burning characteristics. In such cases, auxiliary booster pumps or other equipment may be necessary.

Always possible is the use of the so-called "bottled gases" typified by one sold under the trade name of Pyrofax. Such a gas supply has been found ideal for the flame photometer, since the gas may be supplied to the burner at any desired constant pressure. Suitable gages must be provided to indicate the pressures actually prevailing when the instrument is in operation, so that proper adjustments may be made each time the photometer is used. A 15-inch water pressure gage for city gases, a 5-pound gage for "bottled gas", and a 25-pound gage for the air supply are satisfactory.

BURNERS. The main requirement of the burner is that when supplied with gas and air at constant pressures it shall produce a steady flame.

The temperature of the flame must be high enough to excite the desired metals, primarily the alkali metals, to emit light, but insufficient to excite those which would produce interferences which could not be eliminated by the optical filter system. The Meker-type Fisher burner has been found very satisfactory.

In certain models of the flame photometer, the Fisher blast lamp, which is provided with a pipe connection for introducing air, has proved most advantageous. In this type of flame photometer, all the air supplied to the burner passes through the atomizer. The main advantages of this arrangement are twofold. First, since all the air for the burner may be filtered, the uncontrolled introduction of sodium or potassium in the form of laboratory dust may be avoided. Second, the steadiness of the flame is less influenced by air drafts in the laboratory. One serious drawback, however, does exist in the fact that the pressure

Table III. Determination of Calcium

Solution	Calcium Added	Magnesium Added	Calcium Determined
	P.p.m.	*P.p.m.*	*P.p.m.*
1	100 (used as standard)	0	(100)
2	100	100	100
3	100	500	100
4	100	1,000	95[a]
5	100	10,000	76[a]
6	50	0	50
7	50	500	50
8	50	1,000	47[a]
9	50	10,000	38[a]

[a] In contrast to what might be expected, that the presence of foreign ions might give rise to positive errors, the presence of such ions actually reduces the over-all amount of emitted light. This inhibitory effect seems to be general and must certainly be considered in flame photometry. By using for calibration standard solutions containing approximately the same components as the unknowns, this effect may be minimized.

Table IV. Combinations of Corning Glass Filters for Flame Photometry

Element	Combination
Sodium	3482 and 9780 (H.R. Lantern Shade Yellow + Colorimeter Blue Green)
Potassium	5850 and 2404 (Blue-Purple Ultra + H.R. Dark Red)
Calcium	5120 (2 thicknesses) (Didymium) + liquid filter of concd. HCl + $CuCl_2$
Lithium	2404 + liquid filter of concd. HCl + $CuCl_2$

through the atomizer must be controlled to a greater extent than in the case of the arrangement wherein the burner draws practically all its air in the normal manner. So far, the use of this type of burner has been reserved for industrial applications where the air has been found to be severely contaminated by dust.

A chimney surrounding the flame serves the purposes of protecting the flame from air drafts, providing a simple means for aperturing the flame so that the unsteady light from the grid of the burner and the edges and tail of the flame will not reach the photocell, and eliminating stray light of the laboratory from the optical system. A glass window over the chimney aperture has, on occasion, been found very useful. Sufficient ventilation in the neighborhood of the flame, in the form of grid openings or louvres, is necessary in order to avoid overheating.

ATOMIZERS. The function of the atomizer is to introduce exceedingly fine droplets of the sample in aqueous solution into the air supply for the burner. Since the sample solution may be either acid or alkaline or contain strong oxidizing or reducing agents, the problem of corrosion is important. Atomizers of glass or stainless steel have been found to give very satisfactory service, and it is probable that atomizers made of hard rubber or a suitable resin would also perform well. Two general types of atomizers have been used in the instruments described in this paper.

The first type is made with two capillary tubes sealed into the walls of a glass flask in such a way that their bores are perpendicular to each other. The blast of air from the tip of one capillary causes a suction in the other sufficient to draw the sample through it. The sample thus entering the air stream is broken into fine droplets, the larger of which collect on the walls of the flask and flow into the drain. The smaller droplets, comprising a virtual fog, are carried by the air stream into the burner where they are thoroughly mixed with the normal burner gases. A second type atomizer may be made as shown in Figure 4, using two stainless steel hypodermic needles. This atomizer has the important advantage of allowing for removal of tips for cleaning or changing to different bore sizes, depending upon the viscosity of the sample to be atomized.

A third type, shown in Figure 7, is constructed of two concentric glass tubes. The inner tube, through which the air passes, is constricted to form an orifice about 1 mm. in diameter, while that of the orifice in the outer tube is about 2 mm. The sample is introduced into the annular space between the two tubes through a side arm. These two tubes may be sealed together to form a single unit or joined by means of an interchangeable ground-glass seal. The latter method allows easy cleaning and adjusting of the tips.

As a result of the suction at the orifice of the sample tube, it is possible to introduce the sample in two ways. Instead of pouring the sample into a funnel as shown in Figure 3, it may be sucked upwards from a beaker through a bent capillary sample tube. The suction is sufficient to raise the sample several inches with ease.

OPTICAL SYSTEM. The obvious function of the optical system is to collect the light from the steadiest part of the flame, render it monochromatic, and then to focus it onto the photosensitive surface. Although any type of lens may be used, such as a flask filled with water, Fresnel lenses of heat-resisting glass having high numerical apertures have been found most satisfactory. High optical quality is not essential.

To isolate the desired light, combinations of simple filters have been found satisfactory, the particular filters used depending upon the metal being measured, and the spectral response curve of the detecting device. Table IV lists some of the filter combinations used so far. As the use of the flame photometer is extended, other more refined devices for producing monochromatic light may be required.

PHOTOSENSITIVE DETECTORS. In the flame photometer, any photoelectric device may be used as a detector, provided it has a response in the part of the spectrum to be used, and a sensitivity high enough for the particular task at hand. Photosensitive surfaces are commercially available which possess spectral sensitivities which vary over wide limits. In certain specific applications a choice of surface may be made, so that the detector is highly sensitive to the characteristic radiation emitted by only one of the elements which may be contained in the sample.

A barrier layer photocell was chosen for the photometer shown in Figure 3 because of its simplicity and its favorable performance characteristics. The sensitivity of this type of photocell is low, as compared with phototubes, and its output must be measured with a galvanometer. It has, however, many advantages for this work: it requires no external electrical power supply; its broad spectral response covers adequately the various wave lengths encountered in the usual sample; it is relatively free from annoying drift and fatigue; and its output for a given light input is steady and reproducible. Its chief disadvantage, its high temperature coefficient, may be minimized by placing it at a cool part of the photometer.

Phototubes of many types may be used if provided with suitable power supplies and amplifiers. Electron multiplier tubes, such as the R.C.A. Type 931-A, have been used very successfully in flame photometers. These tubes are extremely sensitive to sodium light and very much less sensitive to that from lithium and potassium, and have the advantage that their output is sufficient to allow the use of rugged types of microammeters or recorders. Their high sensitivity makes possible accurate analytical measurements on solutions containing as little as 0.1 p.p.m. of sodium. One of the main disadvantages of this tube is the fact that a well-regulated power supply is required. Very satisfactory results have been obtained using twenty 45-volt B batteries in series, so wired as to provide 90 volts for each stage of the multiplier. The output of this type of tube at constant light input has been found to fall off for a period of several hours after the power has been applied; accordingly it is not satisfactory for photometers designed for laboratory analyses, which must be ready at all times for immediate use. Once these tubes have reached equilibrium, their output is very steady, and their per-

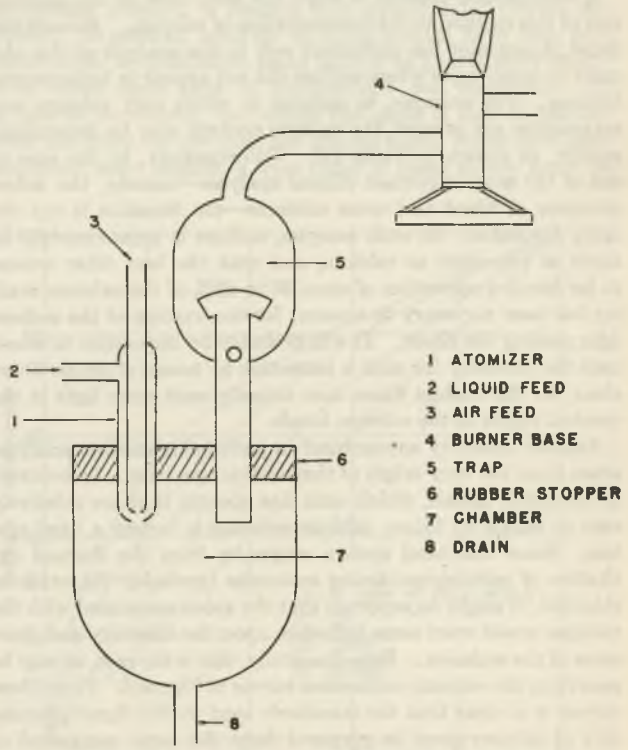

Figure 7. Concentric Tube Type of Atomizing Unit

In using this type of atomizer, all the air for the flame passes through the atomizer

formance entirely satisfactory. A photometer so equipped may be left turned on for very long periods of time, and may thus be used to advantage in installations where analyses are required constantly, three shifts per day.

ELECTRICAL INSTRUMENTS. The particular instrument selected to indicate or record the output of the photoelectric detector will depend in a large measure on the type of detector used and the sensitivity and precision desired.

A multirange meter is convenient if samples of widely different concentrations are encountered, as is also a shunt for varying the individual ranges of the instrument scale. An appreciable period or sluggish response in the meter is advantageous, since it tends to smooth out any slight flicker of the flame. The galvanometer used in Figure 2 is a G.E. Model 32C-245-G9 having a sensitivity of 0.001 microampere per scale division and a period of 4 seconds.

The output of an electron multiplier for solutions containing up to 25 p.p.m. of sodium has been found to be between 10 to 100 microamperes in the various types of flame photometers so far used. This high output permits the use of a rugged pointer-type microammeter. If a potentiometric recorder is used, it is important that it be designed for high damping resistance, so that a certain degree of overdamping is obtained when measurements are made across a fairly high resistance. A micromax with a full-scale range of 100 millivolts, designed for an external circuit resistance of 50,000 ohms, has been used very satisfactorily. With this recorder connected to measure a potential across a 10,000-ohm resistor through which the electron multiplier current is flowing, it is practical to record sodium levels in flowing samples in the range from 5 p.p.m. full scale up to 100 p.p.m. If the 10,000-ohm resistor is variable, the range of the recorder may be varied at will. Another satisfactory method of adjusting the sensitivity range of the photometer when using an electron multiplier is, of course, to vary the voltage applied per stage of the tube until the desired range is obtained.

APPLICATIONS OF FLAME PHOTOMETRY

Generally speaking, the flame photometer may be used wherever the analysis of aqueous samples for their sodium, lithium, potassium, or calcium contents is required. It should be emphasized that this method, as described, is in reality a method for rapidly determining the amounts of metal ions in solutions. Before applying it to the analysis of a wide variety of materials, a certain amount of sample preparation, which on occasion may be tedious and time-consuming, is still essential. In favor of the method, however, is the fact that, since the number of sources of possible interferences is small, procedures for preparing samples may be employed which in general are simpler than those normally used. Specifically, it must be recognized that before applying the method to an entirely new material, a careful investigation should be made to determine the possibly large influence of the foreign ions and molecules present upon the light being used for analysis. It has been noted, for example, that the presence of most foreign salts and acids in a solution being analyzed for sodium, potassium, or calcium tends to lower the amount of light emitted by them. Certain organic molecules tend to lower the amount of light emitted, while others increase the light output. In other cases—for example, the determination of calcium in a solution containing considerable sodium—the readings obtained are high because of inability to filter calcium radiation entirely free of sodium. In most cases, however, where solutions of low sodium and potassium concentrations are analyzed, the accuracy of the results obtained is equal to or better than that given by chemical means, and the speed of the method makes it extremely attractive. The number of applications which have already been made is large and is constantly growing, and at this time only a few will be cited.

Perhaps the simplest and most obvious application is to the rapid or continuous analysis of water samples for sodium, potassium, or calcium, since in general the concentrations of these metals fall within the most useful range of the photometer without dilution. Both recording and indicating instruments have been used satisfactorily. For many years designers of water-softening installations have been faced with the need for an analytical instrument which would indicate within an extremely short time the exhaustion of the zeolite bed, and if possible initiate the regeneration cycle. Since these water softeners operate by introducing sodium ions into the water in proportion to the calcium and/or magnesium removed, the first indication of the exhaustion of the zeolite bed is a drop in the sodium level of the effluent water. Rapid analyses for sodium allow this exhaustion point to be detected with ease. If a recording flame photometer is being used, a sudden drop in the recorded sodium level may be used to actuate the necessary valves for transferring the influent to a previously regenerated bed and to start the regeneration cycle for the exhausted bed.

The same type of installation is suitable for use in determining the sodium levels in waters "demineralized" by synthetic ion-exchange resins.

In many industrial processes where it is necessary to remove sodium salts from precipitated products by washing with water, rapid determination of the sodium content of the influent and effluent waters, or a continuous record thereof, makes for accurate control of the washing cycle. Concentrated solutions such as brines or sea water must usually be diluted prior to analysis.

The flame photometer has a definite place in the general analytical laboratory, for it simplifies many of the usually laborious determinations. Among these might be cited the determination of either sodium or potassium in the presence of the other, and the determination of calcium in the presence of magnesium.

Of frequent and important use to the agronomist is a knowledge of the potassium and sodium content of soil samples. Suitable extraction or other processes for bringing these salts into solution have long been used in connection with standard procedures of soil chemistry. Where large numbers of soil samples must be analyzed routinely, the flame photometer should prove of great value. In connection with studies of plant metabolism and nutrition this same instrument could be used.

Some of the most interesting uses to which these instruments have already been put concern the analysis of biological materials. In the hands of several clinical research workers, the flame photometer has been used successfully to analyze such materials as whole blood, blood serum, urine, body fluids, and tissue residues. These analyses, which are usually both difficult and time-consuming as a result of the very nature of the samples and the small size of the average samples which are available, may readily be performed. For the determination of sodium in blood serum, a sample of as little as 0.1 ml. can be satisfactorily analyzed after diluting by a factor of 1 to 100. The potassium content of serum could conceivably be determined on a 0.1-ml. sample after diluting to 2.5 ml., but a 0.5-ml. sample is much to be preferred. This fact, plus the speed with which the analyses may be completed, makes possible certain studies which have hitherto been impossible. Thus, in the clinical laboratory, the flame photometer has proved itself to be a most valuable instrument in connection with research on metabolic studies, the rapid diagnosis, and the treatment of certain diseases.

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LITERATURE CITED

(1) Barber, H. H., and Kolthoff, I. M., *J. Am. Chem. Soc.*, **50**, 1625 (1928).
(2) Cholak, J., and Hubbard, D. M., IND. ENG. CHEM., ANAL. ED., **16**, 728 (1944).
(3) Griggs, M. A., Johnstin, R., and Elledge, B. E., *Ibid.*, **13**, 99 (1941).
(4) Heyes, J., *Angew. Chem.*, **50**, 871 (1937).
(5) Jansen, W. H., Heyes, J., and Richter, C., *Z. physik. Chem.*, **A174**, 291 (1935).
(6) Lundegårdh, H., "Die quantitative Spektralanalyse der Elemente", Jena, Gustav Fischer, Part I, 1929; Part II, 1934.
(7) Lundegårdh, H., *Lantbruks-Högskol. Ann.* (Sweden), **3**, 49 (1936).
(8) Lundegårdh, H., and Philipson, T., *Ibid.*, **5**, 249 (1938).
(9) McClelland, J. A. C., and Whalley, H. K., *J. Soc. Chem. Ind.*, **60**, 288 (1941).
(10) Mitchell, R. L., *Ibid.*, **55**, 269 (1936).
(11) Schmitt, L., and Breitweiser, W., *Bodenkunde u. Pflanzenernähr*, **9–10**, 750–7 (1938).
(12) Schuhknecht, W., *Angew. Chem.*, **50**, 299 (1937)

Determination of Sulfur Dioxide

Improved Monier-Williams Method

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A modification of the official A.O.A.C. Monier-Williams method is described which possesses a marked increase in sensitivity over the volumetric application of the official method. Comparisons are made with the official method and a modified Bennett-Donovan method using dehydrated vegetable products.

DURING recent studies of the effect of time and temperature on sulfur dioxide levels of sulfited cabbage and carrots conducted in the Subsistence Research and Development Laboratory, it was necessary to utilize a highly sensitive and reproducible method for determining small sulfur dioxide losses. Although the Monier-Williams method (*1*) has been adopted as official by the A.O.A.C., it was not found sufficiently sensitive for the authors' purpose. Methods involving distillation into iodine (*4*) were not considered satisfactory, owing to the volatile sulfur content of cabbage, as they gave abnormally high values on some unsulfited controls.

The modification of the Bennett and Donovan procedure as described by Prater, Johnson, Pool, and Mackinney (*6*) gave rise to considerable difficulty. It was thought that this could undoubtedly be attributed, not to the specificity of the method, but to the conditions under which it was operated in this laboratory. The samples were analyzed every 2 to 4 weeks, and owing to limited personnel, it was not possible to have the same analyst make the determinations each time. As a result, there was usually some uncertainty of end points, particularly the flash end point. A more serious difficulty was the darkening of the vegetables which occurred under the higher storage temperatures and markedly interfered with the observance of the end point. When the Bennett and Donovan method was applied to samples withdrawn periodically from high-temperature storage there were often noted increases and losses in sulfur dioxide levels which could not be accounted for by normal variation within a sample, and over-all trends were difficult to obtain. It is indicated that the authors did not consider the applicability of the method to samples damaged by heat during processing or samples that had been held under storage at elevated temperatures.

In order to satisfy requirements, it became necessary to modify the Monier-Williams method in such manner that its sensitivity would be increased to the same order as that of the modified Bennett-Donovan method when the latter was operated under ideal conditions with normal samples. Nissen and Petersen (*5*) have simplified the official Monier-Williams method in two ways: (1) by neutralizing the 3% hydrogen peroxide to pH 4.0 with sodium hydroxide and (2) by titrating the absorbed sulfur dioxide to pH 4.0 utilizing a glass electrode. Essentially, this speeds up the preparation of the hydrogen peroxide and eliminates the uncertainty of the bromophenol blue end point. Recently Taylor (*7*) reported the method had little advantage over the official method, particularly if gravimetric checks were to be obtained. Obviously, gravimetric checks are too time-consuming for rapid analyses.

There is probably only one fallacy in the volumetric application of the official method and the modified method of Nissen and Petersen, and that lies in the buffering capacity of the absorbed carbon dioxide in the final solution to be titrated. The carbon dioxide serves as a sweeping agent to pass the liberated sulfur dioxide through the condenser into the hydrogen peroxide. An atmosphere of an inert gas also prevents oxidation of the sulfur dioxide. By the simple expedient of substituting nitrogen for carbon dioxide the above conditions can be obtained, but as shown in Figure 1 the buffering action of carbon dioxide is eliminated and the sensitivity of the titration is markedly increased by approximately 18 times. The end point becomes 5.8 to 6.0 instead of 4.0. Although the glass electrode has been used entirely in this laboratory, there is no reason why an indicator titration could not be substituted.

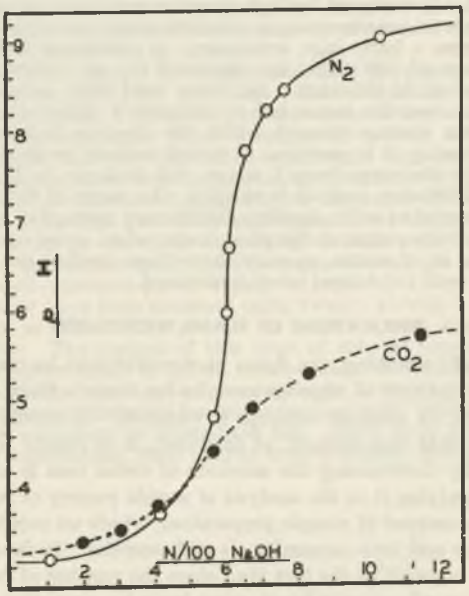

Figure 1. Titration Curves
Carbon dioxide *vs.* nitrogen

The same effect can be obtained by carefully boiling away the carbon dioxide prior to the titration. This, however, adds one more step and creates an added danger in that loss from spattering may occur in routine analyses. In addition, extra time is required, because the solution to be titrated must be brought to the working temperature of the electrodes.

METHOD APPLICATION

APPARATUS. The distillation apparatus, diagrammed in Figure 2, consists of *a*, a 500-ml. wash bottle for pyrogallol reagent; *b*, a 500-ml. wash bottle for water; *c*, a 2-liter, 3-necked distilling flask; *d*, a reflux condenser; *e*, a dropping funnel; and *f*, a receiver consisting of a 125-ml. Erlenmeyer flask and a Peligot tube, *g*. This apparatus is preferred by the authors, but is not mandatory. The one described by the A.O.A.C. (*1*) may be used, but is not considered as convenient.

Titration assembly utilizing a glass and calomel electrode (a Beckman Model G pH meter employing the long electrodes was used by the authors).

REAGENTS. Hydrogen peroxide, 3%, prepared by diluting a 30% stock solution (Superoxal) and adjusting to pH 4.0 with 0.1 *N* sodium hydroxide. It is not necessary to adjust the strength of this reagent to exactly 3% (*5*).

Pyrogallol solution (*8*). Three hundred grams of pyrogallic acid in 1 liter of water; add 2.5 volumes of 50% sodium hydroxide.

Nitrogen, water-pumped, 99.7% pure, commercial cylinder

Hydrochloric acid, concentrated.

Sodium hydroxide, standard 0.010, 0.025, 0.050, and 0.10 *N* solutions.

PROCEDURE. Connect the distilling apparatus as shown in Figure 2, using rubber stoppers throughout. Add to wash bottles *a* and *b* about 400 ml. of pyrogallol reagent and distilled water, respectively. The bottles require only infrequent recharging, as the amount of oxygen present in the nitrogen is very low. Add 15 ml. of the 3% hydrogen peroxide reagent to receiving flask, *f*, and 5 ml. to the Peligot tube, *g*. Connect the receiving flask to the upper end of the condenser, making certain that the delivery tube extends below the surface of the hydrogen peroxide in the receiving flask.

Sweep out the assembled apparatus for approximately 5 minutes with the oxygen-free nitrogen, adjusting the gas so that there is a steady flow through the Peligot tube. Remove the stopper containing the dropping funnel, add the sample, usually 25 grams, and immediately restopper. Avoid adding in such a manner that some of the sample may remain in the neck of the flask. Add 300 ml. of recently boiled and still hot distilled water, containing 10 ml. of hydrochloric acid, through the dropping funnel. The addition must be made steadily and a small portion should be allowed to remain in the dropping funnel to avoid loss of liberated sulfur dioxide through back pressure. Reflux for 1 hour (in the case of dehydrated vegetables) in a current of nitrogen. Shut off the water in the condenser and continue the distillation until the delivery tube from the condenser becomes just warm (this step may be omitted in the case of dehydrated vegetables). Transfer the contents of the receiving flask and Peligot tube to a 250-ml. beaker with adequate washing. Dilute to approximately 100 ml., and titrate with standard alkali to pH 6.0. Choose the normality on the basis of the suspected sulfur dioxide content or the initial pH. Table I gives the approximate initial pH values corresponding to the normalities of the sodium hydroxide to be used. Correct for a reagent blank run in identical manner and calculate as p.p.m. of sulfur dioxide.

Table I. Normality of Sodium Hydroxide to Be Used

(On basis of initial pH of solution to be titrated)

Initial pH	Normality of NaOH	Mg. of SO_2 Equivalent to 1 Ml. of Standard NaOH
<1.9	0.100	3.2
1.9–2.2	0.050	1.6
2.3–2.5	0.025	0.8
>2.5	0.010	0.32

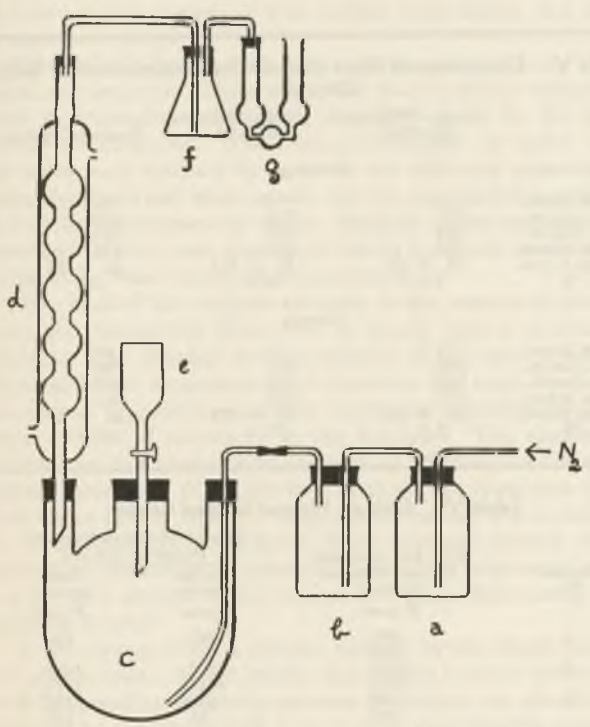

Figure 2. Distillation Apparatus

Table II. Recovery of Sulfur Dioxide Added to Blank Determinations

(Modified Monier-Williams method)

SO_2 Present *Mg.*	SO_2 Recovered *Mg.*	Recovery %
91.12	90.21	99.0
91.30	90.80	99.5
92.26	92.35	100.1
90.10	88.49	96.1
88.54	88.16	99.6
88.54	88.19	99.6
20.86	21.35	102.4
21.09	21.10	100.1
8.32	8.30	99.8
8.22	8.25	100.4
3.97	3.95	99.5
3.96	3.90	98.5

DISCUSSION

The recovery of added sulfur dioxide to aqueous solutions or blank determinations is shown in Table II. Sodium metabisulfite solutions stabilized with sodium pyrophosphate decahydrate were used. The recovery was excellent. Recovery of added sulfur dioxide to cabbage and carrots is shown in Table III. The recovery data are comparable with those reported by Prater and co-workers for the modified Bennett-Donovan method (*6*). It is thought that the extra step involved in the addition of the sulfur dioxide solution, following the addition of the sample, may partially account for the lower results obtained when compared to those previously shown for the aqueous solutions.

Although it has been reported (*3*) that sulfhydryls do not interfere in the Monier-Williams method, it was thought advisable to check it as a possible, but not probable, source of error. Determinations run with added hydrogen sulfide gave titration curves which coincided with those without added hydrogen sulfide. A blank consisting of water saturated with hydrogen sulfide gave a zero titration with 0.01 *N* sodium hydroxide. In the latter case sulfur appeared to be precipitated to some extent in the hydrogen peroxide, but this did not interfere.

To study the effect of varying sample size, a series of 5-, 10-, 15-, 25-, and 50-gram samples were prepared. The samples were ground in the Wiley Mill with a 1.0-mm. screen, utilizing small charges to avoid loss of sulfur dioxide due to heat, until practically all had passed through the screen. Care was taken to blend the charges well. Prepared samples were stored under refrigeration. These precautions were taken in order to avoid the errors due to the possibility of uneven sulfiting and nonuniform particle size, and to ensure reproducibility of results.

The results shown in Table IV indicate that on the basis of the calculated coefficients of variation (percentage of relative variation of the distribution about the mean) utilization of 25- to 50-

Table III. Recovery of Sulfur Dioxide Added to Dehydrated Cabbage and Carrots

(Modified Monier-Williams method)

SO_2 Originally Present *Mg.*	SO_2 Added *Mg.*	SO_2 Found *Mg.*	SO_2 Recovered *Mg.*	Recovery %
		Cabbage		
18.96	14.82	33.70	14.74	99.5
18.96	19.13	37.46	18.50	96.7
18.96	19.90	37.81	18.85	94.7
18.96	19.65	37.74	18.78	95.6
18.96	9.44	28.46	9.50	100.6
22.22	20.22	41.79	19.57	96.8
		Carrots		
22.80	19.49	42.05	19.25	98.8
22.80	9.82	32.26	9.46	96.3
22.77	45.70	66.84	44.07	96.4

Table IV. Effect of Varying Sample Sizes in Determination of Sulfur Dioxide

(Modified Monier-Williams method)

Detn. No.	Sample Weight 50 g.	25 g.	15 g.	10 g.	5 g.
		P.p.m. of Sulfur Dioxide			
		Cabbage			
1	[a]	752	727	770	776
2	...	783	691	789	831
3	...	751	723	665	727
4	...	749	750	803	783
5	...	773	760	672	816
6	...	755	767	707	716
C.V., %[b]	...	1.7	2.8	7.6	5.3
		Carrots			
1	924	909	...	...	...
2	924	905	...	...	...
3	929	916	...	...	...
4	934	901	...	...	...
5	915	925	...	...	...
6	925	903	...	...	...
C.V., %	0.6	0.9	...	...	...

[a] In the case of dehydrated cabbage difficulty was encountered when sample size was increased much in excess of 25 grams owing to charring in flask during distillation.

[b] $C.V. = \left(\frac{\sqrt{\frac{\sum d^2}{n}}}{M}\right) 100$

where

$C.V.$ = coefficient of variation in per cent
d = deviation of individual determinations from the mean, regardless of sign
n = number of determinations
M = mean

gram samples offers the best opportunity of reproducing results. If a large number of samples were run at each level, it is highly probable that the average values would be about the same.

The modified method was compared with both the official and the modified Bennett-Donovan method when used for cabbage and carrots. Table V summarizes comparative data obtained from 37 determinations on a single sample of cabbage and 27 determinations on a single sample of carrots by the three methods. Of the three methods the official A.O.A.C. gave the poorest replication, but consistency of replication was very good for either the modified Monier-Williams or the modified Bennett-Donovan method. The mean value for cabbage obtained by the former is only slightly lower than that found for the latter, while the mean value found for carrots was substantially higher. It was thought that possibly the pigmentation of carrots caused an overtitration of the so-called "flash" end point which would tend to yield lower results by the Bennett-Donovan procedure. The difference, however, was too great to make this assumption entirely logical.

In the absence of unsulfited controls a further check was made on freshly ground raw carrots. Samples were taken of such size to be comparable to 25 grams, in the case of the Monier-Williams methods, and 8 grams, in the case of the Bennett-Donovan method, of 10% moisture products. No attempt was made to remove any of the volatile materials which would be liberated during dehydration. No sulfur dioxide was found by either of the Monier-Williams methods and ±30 p.p.m. were found by the Bennett-Donovan method. Nevertheless, because of the incomplete binding of the acetone and sulfur dioxide in the Bennett-Donovan method as the titration approaches completion, it was suggested (*2*) that glyoxal should be used in place of the acetone. Four milliliters of a 30% solution were found sufficient to bind the sulfur dioxide, and the end point was more persistent and much easier to detect. However, the same sample of carrots yielded similar low results comparable to those obtained when acetone was used, and it was postulated that this particular sample had been heat-damaged in processing.

In support of this assumption three samples of dehydrated carrots, in which heat damage or scorch could not be detected, were analyzed by the modified Monier-Williams procedure and checked by the modified Bennett-Donovan method. Generally good agreement was obtained in all three samples (Table VI). This further emphasizes that the Bennett-Donovan method may not be satisfactory when applied to heat-damaged samples.

The data presented in Table VI may be utilized in explaining the data in Table V for carrots. It has been shown (Table VI) that good agreement is obtained between the modified Monier-Williams and the Bennett-Donovan procedures when normal sulfited carrots are used. It can be anticipated that all three methods would agree on normal samples. The amount of sulfur dioxide absorbed by hydrogen peroxide would not vary on merely changing the carrier gas, as this gas does not enter into the chemical reaction. Although it would appear from Table V that the mean values for carrots when obtained by the Bennett-Donovan and the official Monier-Williams methods are in agreement, it is suggested that this is not the case and that if a large number of determinations had been made by the official method the mean value would have been in agreement with that obtained by the modified Monier-Williams procedure.

Potentiometric curves have been plotted for a series of routine samples, including both those which were normal and obviously scorched or heat-damaged. In no case has there been noted any deviation from the typical curve shown in Figure 1. Any change in the shape of the curve would indicate the presence of substances other than sulfur dioxide in the hydrogen peroxide. This is another indication of the specificity of the method.

SUMMARY

The official A.O.A.C. Monier-Williams method for the determination of sulfur dioxide, though basically correct, is insufficiently sensitive. Its sensitivity may be increased approximately 18 times by eliminating the buffering action of the carbon dioxide in the solution to be titrated. This may be achieved by either employing oxygen-free nitrogen as the carrier gas or eliminating the dissolved carbon dioxide by careful boiling. A modification of the official Monier-Williams method utilizing oxygen-free nitrogen as a carrier gas and potentiometric titration may be applied equally as well to samples of low or high sulfur dioxide

Table V. Comparison of Three Methods for Determination of Sulfur Dioxide

	Monier-Williams Modified	Monier-Williams A.O.A.C.	Bennett-Donovan
	Cabbage		
No. of detns.	15	6	16
Max., p.p.m.	790	866	870
Min., p.p.m.	748	632	710
Mean, p.p.m.	760	804	812
Range, p.p.m.	M ± 21	M ± 117	M ± 80
C.V., %	2.3	9.9	5.8
	Carrots		
No. of detns.	10	7	10
Max., p.p.m.	926	891	730
Min., p.p.m.	901	545	650
Mean, p.p.m.	911	690	691
Range, p.p.m.	M ± 13	M ± 173	M ± 40
C.V., %	0.9	22.5	4.3

Table VI. Data on Normal Sulfited Carrots

Sample No.	SO_2 by Modified Monier-Williams Method	SO_2 by Modified Bennett-Donovan Method: Using acetone	Using glyoxal
	P.p.m.	*P.p.m.*	*P.p.m.*
1	664	600	610
	653		
2	1469	1406	1422
	1471	1414	1414
3	197	122[a]	102
	196	96	130

[a] Titration of less than 1 ml. of 0.05 *N* iodine.

content. It utilizes a fairly large sample size, appears to be specific, and is unaffected by pigments, pigment changes, or sulfhydryls.

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LITERATURE CITED

(1) Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis, 5th ed., pp. 463–4 (1940).
(2) Mackinney, Gordon, University of California, Division of Food Technology, Berkeley, Calif., private communication.
(3) Monier-Williams, G. W., Reports on Public Health and Medical Subjects, No. 43, London Ministry of Health, 1927.
(4) Nichols, P. F., and Reed, H. M., IND. ENG. CHEM., ANAL. ED., 4, 79 (1932).
(5) Nissen, B. H., and Petersen, R. B., *Ibid.*, 15, 129 (1943).
(6) Prater, A. N., Johnson, C. M., Pool, M. F., and Mackinney, G., *Ibid.*, 16, 153 (1944).
(7) Taylor, L. V., *J. Assoc. Official Agr. Chem.*, 27, 386 (1944).
(8) Townley, R. C., and Gould, I. A., *J. Dairy Sci.*, 26, 689 (1943).

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Polarographic Analysis of Aluminum Alloys

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Procedures have been developed for the polarographic determination of iron, copper, lead, nickel, and zinc in aluminum alloys. The alloy is heated with sodium hydroxide and the solution completed in nitric acid. In the absence of chloride, ferric iron and copper give well separated waves. If the ratio of iron to copper is large, the ferric iron is reduced with hydroxylamine hydrochloride. The lead wave is determined after reduction of the ferric iron and precipitation of copper as cuprous thiocyanate and adjustment of the pH. The nickel and zinc waves are determined after adjustment of the pH of the solution of the alloy, and addition of hydroxylamine hydrochloride, thiocyanate, sodium citrate, and pyridine. Alternate procedures are discussed. The proposed procedures give satisfactory results and are especially recommended in routine analysis. The actual time spent in the total analysis for the five elements will be less than 45 minutes in routine work.

IN THIS paper are discussed rapid, reliable methods for the polarographic analysis of iron, copper, lead, nickel, and zinc in aluminum alloys.

The methods developed for iron, copper, and lead are both rapid and accurate. These metals can be determined with ordinary polarographic accuracy in aluminum alloys by the procedure recommended. The method developed for nickel can be made more accurate by more or less elaborate separations from aluminum and other metals, but the recommended method is sufficiently accurate for routine analyses. Two methods are described for zinc; one gives rapid results but is not so accurate as the other, which is more time-consuming.

Since most of the analyses are made in the presence of aluminum, the "supporting electrolyte" is usually high in aluminum content. The diffusion current constant of the various metals depends on the concentration of aluminum and must be determined in a supporting electrolyte containing approximately the same amount of aluminum as the unknown. The aluminum content can be varied at least 5% without affecting the diffusion current constant. If, in the final analysis, the aluminum content varies greatly from the usual concentration, it is necessary to adjust the aluminum content of the unknown solution or to determine the diffusion current constant in this solution by adding a known amount of the element and again determining the diffusion current.

In the present work, a uniform method for the dissolution of the alloy is used. Stated briefly, this method involves treatment with 20% sodium hydroxide solution to dissolve the aluminum, followed by treatment with 1 to 1 nitric acid (specific gravity 1.22). Hydrochloric acid is avoided, since it interferes in the determination of iron and copper. Chloride ion gives an anodic wave beginning at around 0 volt (*vs.* S.C.E.), so that the iron wave, shifted to this potential, coincides with the copper wave in solutions containing chloride.

Synthetic aluminum alloy solutions were prepared from solutions of the following: 99.9% pure aluminum metal obtained from the Aluminum Company of America, Baker and Adamson nickel shot, the solution of which was analyzed for nickel by precipitation with dimethylglyoxime, Baker standardizing zinc foil, Baker standardizing iron wire, Baker electrolytic copper foil, and Merck reagent lead nitrate. The aluminum solution was prepared by the same procedure as employed with alloys (Section 2). The other metals were dissolved in a small volume of 1 to 1 nitric acid and diluted with water to make solutions containing 1.00 mg. per ml. or other appropriate amounts.

Polarographic measurements were made with both a Sargent-Heyrovský Model XI polarograph and a manual apparatus.

Oxygen was removed from the polarographic solutions by bubbling with nitrogen gas which was purified by passing through solutions of chromous chloride, sodium hydroxide, and water.

The temperature used in all this work was 25.0° C. The chemicals used were of reagent grade or better. The polarographic cells were of the type described by Hume and Harris (*1*). Tube *D* has a 1-cm. fine sintered-glass disk at the end. The agar bridge, *C*, should be inserted into tube *D* just before the polarogram is run to prevent the diffusion of potassium chloride from the agar bridge into the polarographic solution. This precaution is important in the determination of iron, since chloride interferes in the separation of the polarographic waves of iron and copper.

All potentials in this paper refer to the saturated calomel electrode.

Polarographic Determination of Iron, Copper, Lead, Nickel, and Zinc in Aluminum Alloys

1. SOLUTIONS REQUIRED. Bromocresol Green (0.1%). To 100 mg. of bromocresol green (tetrabromo-*m*-cresol-sulfonphthalein) in an agate mortar add 2.9 ml. of 0.05 *M* sodium hydroxide. Rub the solid until dissolved and dilute with water to 100 ml.

Dithizone (0.05% solution in carbon tetrachloride). Dissolve 500 mg. of dithizone (diphenylthiocarbazone) in 1000 ml. of carbon tetrachloride (U.S.P.).

Gelatin (0.5%). Soak 0.5 gram of gelatin in 100 grams of distilled water. Heat to the boiling point, cool to room temperature, and add a few drops of toluene as a preservative.

Hydrochloric Acid (0.1 *M*). Dilute 8.5 ml. of constant-boiling acid to 500 ml.

Hydroxylamine Hydrochloride (2 *M*). To 13.9 grams of hydroxylamine hydrochloride (recrystallized from water) add distilled water to make 100 ml.

Nitric Acid (1 to 1 or sp. gr. 1.22). Mix 500 ml. of concentrated nitric acid (sp. gr. 1.42) with 500 ml. of distilled water.

Potassium Thiocyanate (2 *M*). To 19.4 grams of reagent potassium thiocyanate, add distilled water to make 100 ml. of solution.

Sodium Citrate (1.25 *M*). To 184 grams of reagent sodium citrate dihydrate add distilled water to make 500 ml. of solution.

Sodium Citrate (saturated). Dissolve 400 grams of reagent sodium citrate dihydrate in 600 ml. of distilled water.

Sodium Hydroxide (1 *M*). To 5 ml. of saturated sodium hydroxide add distilled water to make 100 ml.

Sodium Hydroxide (15%). Dissolve 150 grams of reagent sodium hydroxide pellets in 850 ml. of distilled water.

Thymol Blue (0.1%). Rub 100 mg. of thymol blue (thymolsulfonphthalein) with 4.3 ml. of 0.05 *M* sodium hydroxide in an agate mortar. After the solid dissolves, dilute with water to 100 ml.

2. Preparation of Alloy Solutions. A. *Method.* To 1.000 gram of aluminum alloy drillings in a 150-ml. beaker covered with a watch glass, slowly add 11 ml. of 15% sodium hydroxide. After the reaction subsides, heat the solution to effect completion of the reaction. Add 20 ml. of 1 to 1 nitric acid, stir, and heat to boil out the lower oxides of nitrogen. The alloy dissolves completely except for a little white residue which is probably metastannic acid and silica. Cool the solution to room temperature and dilute to 50.0 ml. in a volumetric flask. For the analyses, use aliquot samples of this solution.

B. *Pure Aluminum Solution.* Prepare an aluminum solution following the procedure outlined above, using pure aluminum in place of the alloy. This solution is used to prepare standard samples for determining the residual current and the diffusion current constants in the various procedures. In the authors' work, a pure aluminum solution was used to prepare synthetic alloy solutions for testing the procedures.

RECOMMENDED PROCEDURES

3. Polarographic Determination of Iron and Copper without Separation. Pipet a 10-ml. aliquot sample of the aluminum alloy solution in nitric acid (Section 2) to a 25-ml. volumetric flask, add 0.5 ml. of 0.5% gelatin solution, and make up to volume. Transfer the solution to a polarographic cell, remove the oxygen from the solution by bubbling with nitrogen gas, and run a polarogram or measure the diffusion current at +0.15 and −0.15 volt (*vs.* S.C.E.). Correct the observed diffusion current for the residual current of a pure aluminum solution: i_d (corrected) = i_d (observed) − i (residual). Compare the corrected diffusion current at +0.15 volt with that of a standard sample prepared with pure aluminum and standard iron and copper to obtain the amount of iron in the sample. Obtain the diffusion current for copper by subtracting the diffusion current at +0.15 volt from that at −0.15 volt (*vs.* S.C.E.), making corrections for the residual current. Compare this diffusion current for copper with that of a standard sample to obtain the amount of copper in the sample.

If the concentrations of iron and copper are too great and the total diffusion current is greater than ca. 20 microamperes, use a smaller aliquot of alloy solution. Add a volume of pure aluminum solution such that the total volume of alloy and pure aluminum solutions is 10 ml.

4. Polarographic Determination of Copper in Presence of Much Iron. To a 10-ml. aliquot sample of the aluminum alloy solution (Section 2) in a 50-ml. beaker, add 0.3 ml. of 2 *M* hydroxylamine hydrochloride solution and heat to the boiling point. Cool to room temperature and transfer quantitatively to a 25-ml. volumetric flask. Add 0.5 ml. of 0.5% gelatin and dilute to the volume mark with distilled water. Transfer the solution to a polarographic cell and bubble with nitrogen gas for 5 to 10 minutes to remove oxygen. Record the polarogram from +0.2 to −0.2 volt or observe the current at +0.15 and −0.15 volt (*vs.* S.C.E.). Correct the diffusion current for the residual current and calculate the amount of copper present by comparing the corrected diffusion current with that observed with a standard sample. It is not strictly necessary to make the measurements at +0.2 or +0.15 volt. The authors recommend that this be done, because in rapid routine work a trace of ferric iron still may be in the solution, giving a diffusion current at +0.2 volt.

5. Polarographic Determination of Lead in Presence of Much Copper and Iron. A. *Method.* Pipet a 10-ml. aliquot of aluminum alloy solution (Section 2) into a 25-ml. volumetric flask. Add a drop of 0.1% thymol blue indicator, then 1 *M* sodium hydroxide until the indicator changes to an orange color, and add 1 ml. in excess. Add 0.5 ml. of 2 *M* hydroxylamine hydrochloride and 0.5 ml. of 2 *M* potassium thiocyanate and shake gently until the red color of the iron thiocyanate disappears. Add a little water to wash down the side of the flask, 0.5 ml. of 0.5% gelatin solution, and dilute the solution to the volume mark with distilled water. Transfer the solution to a polarographic cell and bubble with nitrogen gas for 5 to 10 minutes to remove oxygen. Record a polarogram from −0.2 to −0.6 volt (*vs.* S.C.E.) or measure the current at −0.3 and −0.5 volt (*vs.* S.C.E.). Correct the observations for the residual current and compare the corrected diffusion current with that of a standard sample.

B. *Alternate Method.* Extract the lead with dithizone (see Section 8 for details).

6. Rapid Method for Determination of Nickel and Zinc. Pipet a 15-ml. aliquot sample of aluminum alloy solution (Section 2) into a 50-ml. volumetric flask, add 5 ml. of 1 *M* sodium hydroxide, and mix thoroughly to make the precipitated aluminum hydroxide redissolve. Add 0.70 ml. of 2 *M* hydroxylamine hydrochloride and 0.50 ml. of 2 *M* potassium thiocyanate and rinse down the neck of the flask with distilled water. Shake the solution in the flask until the red color of iron thiocyanate disappears; then add 5.0 ml. of 1.25 *M* sodium citrate solution. Mix the solution thoroughly and add 0.50 ml. of pyridine, 2 drops of 0.1% bromocresol green indicator, and 15% sodium hydroxide until the indicator changes to a distinct green color (pH = 4.5). Add 1.0 ml. of 0.5% gelatin solution and dilute the solution to the volume mark with distilled water. Transfer the solution to a polarographic cell, bubble with nitrogen gas for 5 to 10 minutes and record a polarogram from −0.4 to −1.4 volts (*vs.* S.C.E.). Compare the nickel and zinc diffusion currents with those observed with standard samples in pure aluminum treated in the same way to determine the amount of nickel and zinc present in the unknown.

7. Separation Method for Determination of Nickel. Electrolyze a suitable aliquot portion of the aluminum solution in nitric acid to remove copper and reduce iron. Transfer the solution to a 50-ml. volumetric flask, add 10 ml. of pyridine, dilute to the volume mark with distilled water, and shake vigorously. Filter the solution through a clean, dry funnel fitted with a coarse-porosity paper—e.g., S. and S. No. 589 "Black Band". Pipet a 20-ml. aliquot of the filtrate and 0.5 ml. of 0.5% gelatin to a 25-ml. volumetric flask and dilute to the volume mark. Transfer the solution to a polarographic cell, bubble with nitrogen for 5 to 10 minutes to remove oxygen, and record a polarogram from −0.6 to −1.0 volt (*vs.* S.C.E.). Compare the diffusion current with that of a standard sample to determine the amount of nickel present.

8. Separation Method for Determination of Zinc. To facilitate the dithizone extraction of zinc, remove copper from the alloy solution before the extraction. Copper can be removed very simply by treating an aliquot portion of the alloy solution with hydroxylamine hydrochloride and potassium thiocyanate or a weighed sample of the alloy may be dissolved in hydrochloric acid. The amount of alloy sample used does not affect the diffusion current constant as in the case of the iron and copper determination, since the zinc is extracted from the solution. For most alloys a 15-ml. aliquot sample of its solution can be treated with 0.5 ml. of 2 *M* hydroxylamine hydrochloride and 0.5 ml. of 2 *M* potassium thiocyanate to remove the copper (the copper thiocyanate precipitate need not be removed from the solution). For alloys of low zinc content—e.g., A.R.I. sample 40—it is convenient to dissolve a 1-gram sample in 25 ml. of constant-boiling hydrochloric acid and filter the solution to remove the residue of copper, nickel, etc.

To the resulting copper-free solution, add thymol blue indicator, 10 ml. of saturated sodium citrate solution, and ammonia until the indicator changes color to a greenish yellow. Transfer this solution quantitatively to a separatory funnel (the stopcocks of the separatory funnels should not be greased but wetted with water). Add 10 ml. of 0.05% dithizone in carbon tetrachloride and shake for 1 to 2 minutes. (If appreciable amounts of lead and zinc are present, the carbon tetrachloride layer becomes a bright cherry red.) Withdraw the carbon tetrachloride phase into a 125-ml. separatory funnel, add 10 ml. of 0.05% dithizone in carbon tetrachloride to the aqueous solution, and extract as outlined above. Continue the extraction until the carbon tetrachloride phase remains green or becomes a brownish purple, then extract once more to ensure the complete extraction of zinc and lead. (If a brownish purple scum forms over the carbon tetrachloride during the extractions, do not transfer it to the 125-ml. separatory funnel. The scum is nickel dithizonate which is slightly soluble in carbon tetrachloride.) Shake the aqueous solution with two small portions of carbon tetrachloride to rinse out droplets containing zinc and lead. Add these to the 125-ml. separatory funnel and discard the aqueous solution. Add 25 ml. of water containing 1 drop of ammonia to the combined extracts and shake to wash the carbon tetrachloride. Withdraw the carbon tetrachloride phase to another 125-ml. separatory funnel, shake the ammonia solution with two small portions of carbon tetrachloride, and add these extracts to the main carbon tetrachloride extract. Discard the ammonia solution and clean the funnel.

To the combined extract add 20 ml. of 0.1 *M* hydrochloric acid and shake for 2 to 3 minutes. (The carbon tetrachloride layer will turn green if no copper is present.) Withdraw the

carbon tetrachloride phase to the other 125-ml. separatory funnel. Rinse the hydrochloric acid solution with two small portions of carbon tetrachloride and combine the latter with the carbon tetrachloride extract. Leave the hydrochloric acid solution in the separatory funnel with any scum and droplets of carbon tetrachloride.

Add 10 ml. of fresh 0.1 *M* hydrochloric acid to the carbon tetrachloride extract, shake for 2 to 3 minutes, and discard the carbon tetrachloride phase. Rinse the hydrochloric acid solution with two small portions of carbon tetrachloride and transfer it quantitatively to the separatory funnel containing the 20-ml. portion.

Shake the combined hydrochloric acid solution with several small portions of chloroform until the chloroform remains colorless, and discard the chloroform. (The nickel dithizonate scum is soluble in chloroform and thus only a few droplets of clear chloroform should remain with the acid solution.) Transfer the acid solution quantitatively to a 100-ml. beaker and boil on a hot plate to expel all the chloroform. Cool the solution to room temperature, transfer it to a 50-ml. volumetric flask, add 1 ml. of 0.5% gelatin and 1 ml. of pyridine, and dilute to the volume mark with distilled water. Transfer the solution to a polarographic cell, bubble with nitrogen for 5 to 10 minutes to remove oxygen, and record a polarogram from −0.2 to −0.6 volt *vs.* S.C.E. to determine lead, and from −0.8 to −1.2 volts *vs.* S.C.E. to determine zinc. Compare the diffusion currents for lead and zinc with that observed for a standard sample prepared by diluting known amounts of lead and zinc solutions, 30 ml. of 0.1 *M* hydrochloric acid, 1 ml. of pyridine, and 1 ml. of 0.5% gelatin to 50 ml.

Discussion

Generally, if we have a mixture of various elements, the accuracy of the determination of the polarographic wave of the element reduced first is not affected by the amount of the other elements present. Thus, a trace of ferric iron can be determined polarographically in the presence of all the other elements considered in this paper.

We now consider the determination of copper in the presence of ferric iron. The first wave corresponds to the reduction of ferric to ferrous iron and the second to the reduction of cupric copper to the metallic state. If the concentration of copper is of the same order of magnitude or greater than that of iron, the accuracy of the copper determination is not affected appreciably by the iron. On the other hand, if the concentration of copper is roughly less than one tenth that of iron, the accuracy of the polarographic copper determination becomes much less than when the iron is absent. This situation often prevails in the analysis of aluminum alloys. In order to get reliable results for copper, it is necessary to make the iron harmless.

A similar situation holds for lead. Aluminum alloys, as a rule, contain very small amounts of lead as compared to iron and copper; therefore, ferric iron and copper must be made harmless before determining the lead polarographically. A similar situation exists for nickel and zinc. Iron and copper must be made harmless before determining nickel and zinc.

The polarographic behavior of some metals is altered greatly in the presence of the high concentrations of aluminum found in solutions of alloys—for example, in the absence of aluminum, nickel and zinc can be determined very simply by using a medium containing thiocyanate. However, with a large amount of aluminum, the nickel wave is distorted, which makes the determination of nickel and zinc impossible. As another example, zinc and nickel, in the presence of much citrate and a little thiocyanate and pyridine, do not give polarographic waves but when much aluminum is present, well-defined waves are obtained. It is found, also, that the diffusion coefficients of ions vary with moderate variations in the aluminum concentration.

Because of these peculiarities, it is necessary to use solutions of the metals containing aluminum at the approximate concentration present in alloy solutions for comparison.

COMBINATION OF IRON, COPPER, AND LEAD

The determination of iron in nitric acid solutions entails no difficulty through interferences by other metals in the solution. However, it is necessary that the aluminum content of the unknown solution be about equal to that of the standard solution, that the solution remain free of chloride, and that a polarographic cell with an outside reference electrode be used (ferric iron oxidizes mercury).

When the amount of copper in the alloy is much less than that of iron, the iron is rendered harmless either by reduction to the ferrous state or by removal from the solution. Reduction can be accomplished simply by adding hydroxylamine hydrochloride to the solution. The reduction in acid solution proceeds very slowly at room temperature and thus the solution should be heated. Copper is not reduced by hydroxylamine hydrochloride and the small amount of chloride introduced with the hydroxylamine is not sufficient to alter the polarographic wave of copper.

In the determination of lead, large amounts of iron and copper interfere, since their waves precede that of lead. Generally, in aluminum alloys, the amounts of iron and copper are much larger than that of lead and these interfering substances must be rendered harmless. This is accomplished most simply by reduction with hydroxylamine hydrochloride in the presence of thiocyanate; iron is reduced to the ferrous state and copper precipitates as cuprous thiocyanate. When this method was applied to some standard aluminum alloys, it was found that the observed lead content was much higher than the reported value—for example, sample A.R.I. 39 showed 0.57% lead as compared to the reported value of 0.49%. If the alloy solution in nitric acid is neutralized to around pH 2.5 before it is treated with hydroxylamine hydrochloride and thiocyanate, the results for the lead determination agree well with the reported values. The adjustment of the pH has the further advantage that in solutions with pH around 2.5, ferric iron and copper are reduced rapidly at room temperature by hydroxylamine and thiocyanate. This is the method recommended for the determination of lead (cf. Section 5).

The high results for lead which are observed in the acid alloy solutions are probably due to the incomplete reduction of copper or to the solubility of cuprous thiocyanate which is reduced at the dropping mercury electrode in the presence of thiocyanate at −0.39 volt. When the pH is adjusted to 2.5 the reduction of copper by hydroxylamine and thiocyanate is complete and the interference with lead is eliminated. The high results for lead were at first attributed to the possible presence of some tin, but it was found that stannic tin does not interfere with the lead determination. However, the presence of tin gives a slight increase in the diffusion currents of both nickel and zinc in the medium in which the pH is adjusted.

Other methods for determining lead were tried without success. In an alkaline cyanide-citrate medium, copper gives no wave but iron gives a wave which coincides with that of lead (dissolution potential of mercury). Since ferric iron cannot be reduced quickly and completely in this medium, the method was abandoned as unsuitable for determining lead.

Lead in sodium hydroxide solutions gives a good wave but some lead is coprecipitated with ferric hydroxide in alloy solutions and no further work was done in this medium.

Other methods beside the hydroxylamine-thiocyanate method may be used to remove iron and copper. By electrolysis, copper is plated out and ferric iron is reduced in acid solutions of the alloys. This method, however, requires a long time (30 to 45 minutes).

Ferric iron is reduced to the ferrous state and copper is precipitated and removed as cuprous thiocyanate when an alloy solution containing thiocyanate is passed through a silver reductor. This method is not recommended in the present work for the reduction of iron and removal of copper, since the volume of solution is increased by rinsing out the reductor. Where this increase in volume is of little consequence, this method is excellent for quickly reducing iron and removing copper.

Copper and small amounts of ferrous iron can be extracted by

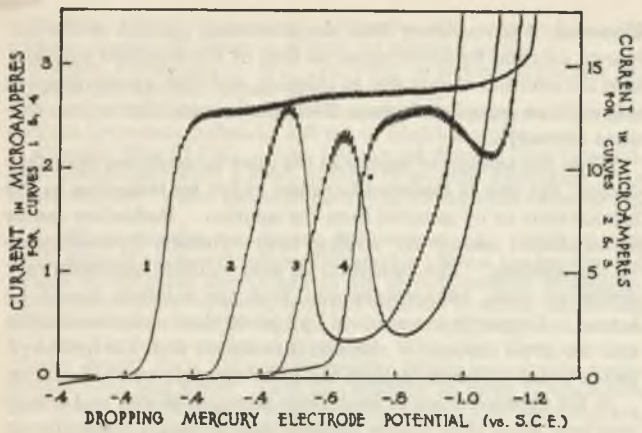

Figure 1. Behavior of Nickel in Thiocyanate Medium

1. Solution containing 1.00 ml. of 0.01 *M* $Ni(NO_3)_2$, 1.0 ml. of 2 *M* KCNS, and 0.5 ml. of 0.5% gelatin, made up to 25.0 ml.
2. Same as 1, but 0.05 ml. of 1:1 nitric acid added before dilution to 25.0 ml.
3. Same as 1, but 5.0 ml. of standard aluminum solution added before dilution to 25 ml.
4. To ca. 20 ml. of solution 3, 2.0 ml. of 1 *M* sodium hydroxide added to neutralize the excess acid. Aluminum hydroxide begins to precipitate

a chloroform solution of α-nitroso-β-naphthol from aqueous solutions buffered with acetic acid and sodium acetate. Ferric iron precipitates with this reagent and is not extracted readily. For use in routine analyses, this extraction method is tedious and not very satisfactory.

From solutions as acid as 0.1 to 0.2 *M* in hydrochloric acid, copper is extracted by dithizone in carbon tetrachloride. From neutral or slightly alkaline solutions, other metals—e.g., zinc and lead—are also extracted but copper can be separated from these metals by shaking the carbon tetrachloride extract with 0.1 *M* hydrochloric acid. Copper dithizonate remains in the carbon tetrachloride phase while the zinc and lead dithizonates enter into the aqueous phase. Iron is not extracted, but large amounts of ferric iron interfere by oxidizing the dithizone. With large amounts of copper, the method is difficult, expensive, and impractical, since large amounts of dithizone and carbon tetrachloride are required.

Copper-free solutions can be obtained by dissolving the alloy in dilute hydrochloric acid. Most of the copper and nickel remain in the residue. Iron dissolves and is present in the ferrous state. This method can be used to dissolve alloys in which zinc is determined by the dithizone extraction method (cf. Section 8).

NICKEL

When an aluminum alloy solution is analyzed, the nickel wave in noncomplex-forming supporting electrolytes cannot be used, since the zinc wave interferes. The wave for nickel in the presence of thiocyanate, which is suitable for determining nickel alone, cannot be used, since it is distorted by a large excess of aluminum. This effect is shown in Figure 1. Nickel in neutral thiocyanate gives a good wave (Figure 1, curve 1). The addition of aluminum produces an apparent maximum in this wave (curve 3) which is due to the presence of too large a concentration of hydrogen ions (compare with curve 2). However, even after the pH is adjusted, the nickel wave in the presence of excess aluminum is distorted and cannot be used for quantitative purposes (curve 4).

In a solution containing either potassium citrate or potassium oxalate as the supporting electrolyte, nickel does not give a polarographic wave. If an excess of ammonia is added to either of these solutions, a wave is obtained with the same half-wave potential as is observed with ammonia and ammonium chloride as the supporting electrolyte. However, if a large amount of aluminum is added to this ammoniacal citrate solution, the nickel wave becomes poorly defined and the diffusion current falls.

If ammonia is used in the absence of citrate to precipitate the aluminum and other trivalent cations in the alloy solution, some of the nickel coprecipitates. In an ammoniacal medium containing ferrous iron, the wave plateau for nickel is not reached completely before ferrous iron begins to be reduced. This makes the measurement of the nickel wave difficult. For these reasons, the determination of nickel after precipitating the aluminum with ammonia is unsatisfactory.

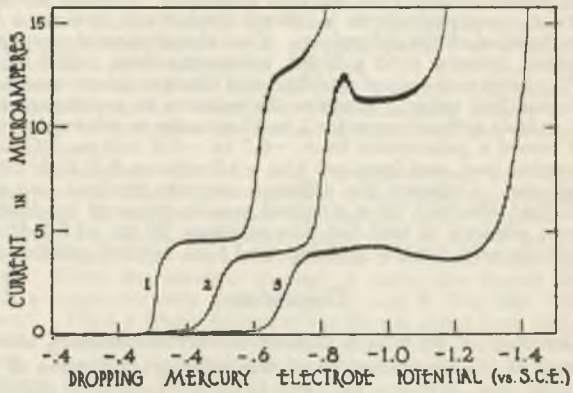

Figure 2. Behavior of Nickel in Citrate-Thiocyanate Medium

1. 15.0 ml. of standard aluminum solution + 4.00 mg. of zinc + 2.27 mg. of nickel run according to recommended rapid procedure for determination of nickel and zinc
2. Same as 1, but pyridine omitted
3. Same as 2, but in absence of zinc

If pyridine is used in place of ammonia to precipitate the trivalent cations, nickel does not coprecipitate. In this medium, nickel gives a well-defined wave with the half-wave potential sufficiently positive to eliminate interference by ferrous iron. This is a satisfactory method for determining nickel in aluminum alloys; however, the thiocyanate used in making ferric iron and copper harmless interferes because of precipitation of nickel pyridine–thiocyanate. For this reason, the hydroxylamine hydrochloride–thiocyanate method recommended for removing copper and reducing iron cannot be used. The removal of copper and reduction of ferric iron can be accomplished by electrolysis.

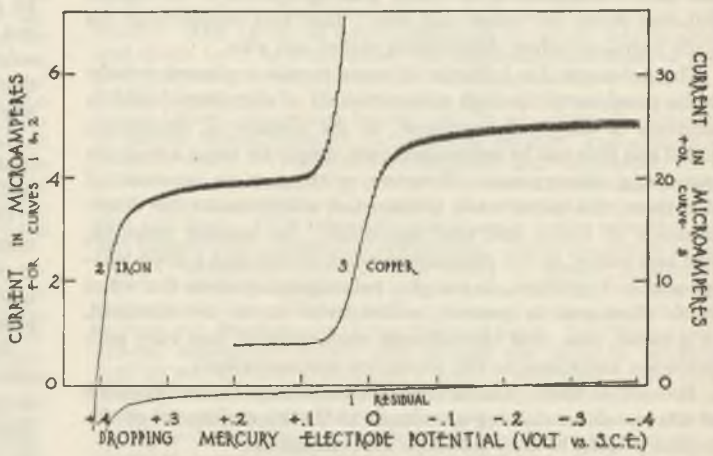

Figure 3. Standard Iron and Copper

1. 10.015 ml. of standard aluminum solution + 0.5 ml. of 0.5% gelatin made up to 25.0 ml.
2 and 3. 10.015 ml. of standard aluminum solution + 5.00 mg. of Cu(II) + 2.00 mg. of Fe(III) + 0.5 ml. of 0.5% gelatin made up to 25.0 ml.

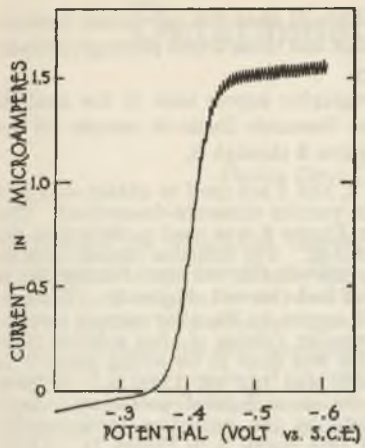

Figure 4. Standard Lead

10.015 ml. of standard aluminum solution + 12.7 mg. of Cu(II) + 2.23 mg. of Fe(III) + 1.00 mg. of Pb(II) run according to recommended procedure (treatment with KCNS, $NH_2OH.HCl$, and NaOH)

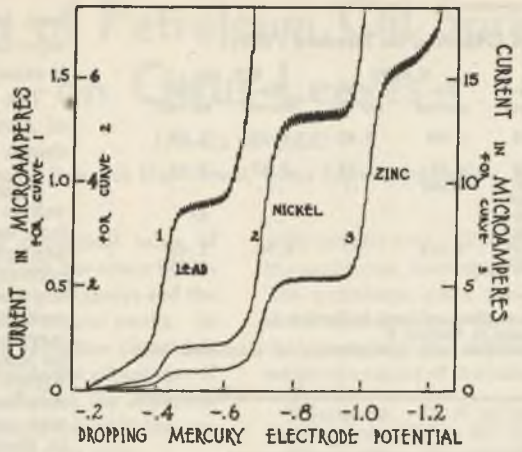

Figure 5. Standard Nickel and Zinc

15.0 ml. of standard aluminum solution + 2.23 mg. of Fe(III) + 12.7 mg. of Cu(II) + 1.00 mg. of Pb(II) + 2.27 mg. of Ni(II) + 5.01 mg. of Zn(II), run according to recommended rapid procedure for determination of nickel and zinc

This is the special method suggested for the separation analysis of nickel (cf. Section 7).

Well-defined nickel waves are obtained in aluminum solutions having a pH around 4.5 and containing a large amount of citrate with some thiocyanate and pyridine (see Figure 2, curve 1). The presence of the citrate prevents the precipitation of nickel with the combination of thiocyanate and pyridine. Use of this fact is made in the recommended rapid method for determining nickel and zinc (see Section 6). It was found that the presence of large amounts of aluminum is necessary to get a well-defined nickel wave. In the absence of much aluminum, no nickel wave is obtained and the zinc wave is distorted. The explanation of this peculiar behavior undoubtedly is that in the absence of much aluminum, the citrate forms stable complexes with nickel and zinc and thus shifts their reduction potentials to values more negative than the hydrogen discharge potential in this medium. The presence of much aluminum ties up much of the citrate in the form of a complex, thus decreasing the stability of the nickel and zinc complexes and making possible the reduction of nickel and zinc.

If the pyridine is omitted in this procedure, the plateau of the nickel wave is distorted, as is shown in curve 3 in Figure 2. [In the presence of zinc the distortion of the nickel plateau manifests itself in the zinc wave (see curve 2, Figure 2)].

ZINC

Since polarographic waves of nickel in various media are abnormal, the determination of zinc in the presence of nickel is limited to solutions in which nickel behaves normally. When the ratio of nickel to zinc is large, a separation is necessary.

In either ammonia or pyridine solutions, zinc in alloy solutions is coprecipitated strongly with the trivalent-cation hydroxides. Zinc is also coprecipitated with ferric hydroxide in solutions containing excess sodium hydroxide.

If citrate is used to keep aluminum in solution, a well-defined zinc wave is obtained in the presence of pyridine at a pH of 4.5 with and without thiocyanate present. Use of this fact is made in the recommended rapid method for determining nickel and zinc (cf. Section 6).

Zinc can be separated readily from most of the metals present in aluminum alloys, by extraction with dithizone in carbon tetrachloride. A procedure was developed and is recommended as an accurate method for determining zinc (cf. Section 8).

A weakly ammoniacal (pH 8.5 to 9) solution of an aluminum sample to which sodium citrate has been added to prevent the precipitation of aluminum and iron, etc., is shaken with a carbon tetrachloride solution of dithizone. Of the metals normally present in aluminum, only copper, lead, zinc, and nickel are extracted from the ammoniacal solution by the carbon tetrachloride solution of dithizone. Nickel dithizonate is slightly soluble in carbon tetrachloride and is extracted slowly after the other metals are completely extracted. When the carbon tetrachloride extract is shaken with 0.1 *M* hydrochloric acid, lead and zinc dithizonate are decomposed and the ions go into the aqueous phase. Copper dithizonate is stable in the presence of the acid solution, and nickel dithizonate decomposes slowly. Therefore, when an ammoniacal citrate solution of aluminum is extracted with dithizone in carbon tetrachloride and the extract shaken with acid as described above, lead and zinc are separated from all other metals.

Colorimetric Determination of Zinc. It should be possible to use a slight variation of this method as the basis for a colorimetric method for determining traces of zinc in aluminum. To the 0.1 *M* hydrochloric acid solution containing zinc and lead, add an acetic acid–sodium acetate buffer to give a pH ca. 4.75 and sodium thiosulfate to form complexes with lead and traces of nickel which may be present. Shake this solution with a standard dithizone solution in carbon tetrachloride and meas-

Table I. Reported Analyses of Alloy Standards

	A.R.I. 39[a]	A.R.I. 40[a]	B.S. 85[b]	B.S. 86b[b]
Copper	7.39	0.37	4.11	7.87
Iron	1.01	0.83	0.39	1.53
Zinc	2.20	0.25	0.014	1.50
Manganese	0.21	0.09	0.56	0.013
Silicon	1.88	5.57	0.46	0.47
Magnesium	0.21	0.13	0.40	...
Nickel	0.83	0.10	...	...
Tin	1.05	0.12	0.01	...
Lead	0.49	0.10	0.007	...
Titanium	0.09	0.07	0.022	0.032
Chromium	0.36	0.09	...	...
Calcium	0.05?	0.05?	...	...

[a] Alloy standards of Aluminum Research Institute.
[b] Alloy standards of National Bureau of Standards.

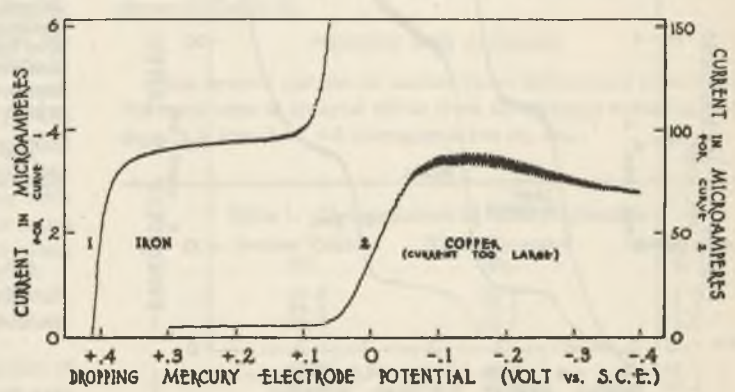

Figure 6. Determination of Iron and Copper in Sample A.R.I. 39

10.015 ml. of A.R.I. 39 solution run according to recommended procedure

Table II. Summary of Results Observed on Standard Alloys

	A.R.I. 39		A.R.I. 40		B.S. 85		B.S. 86b	
	Reported	Observed	Reported	Observed	Reported	Observed	Reported	Observed
Iron	0.98 to 1.01[a]	0.98	0.83	0.84	0.39	0.40	1.53	1.56
Copper	7.39[a]	7.62	0.37	0.39	4.11	4.18	7.87	7.94
Lead	0.49	0.47 0.45[b]	0.10	0.11 0.09[b]	0.007	...	...	...
Nickel	0.83	0.80[c] 0.85[d]	0.10	0.10[c] 0.09[d]	...	...	...	0[a]
Zinc	2.20	1.98[c] 2.15[b]	0.25	0.24[c] 0.22[b]	0.014	...	1.50	1.42[c] 1.45[b]

[a] Not officially accepted values.
[b] Results obtained by dithizone extraction method outlined in Section 8.
[c] Results obtained by rapid method outlined in Section 6.
[d] Results obtained after electrolysis of solution and precipitation of aluminum with pyridine. Method in Section 7.

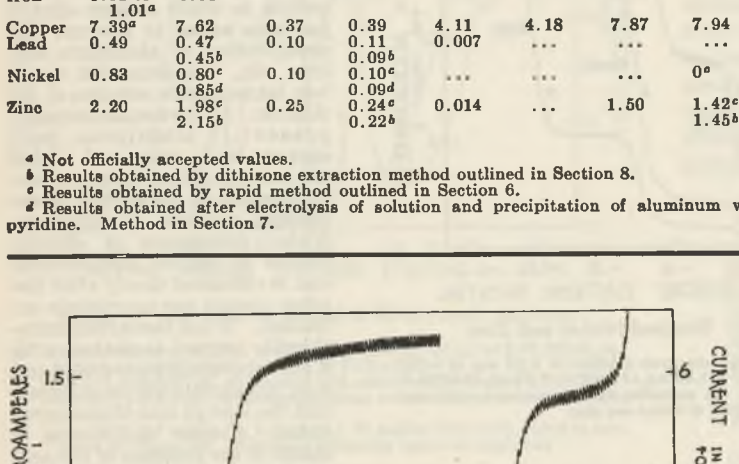

Figure 7. Determination of Copper and Lead in Sample A.R.I. 39

1. 1.000-ml. aliquot sample
2. 20.015-ml. aliquot sample

ure the transmittancy of the carbon tetrachloride phase with light of either 520 to 540 or 620 mμ wave length (*2*).

APPLICATION OF RECOMMENDED PROCEDURE TO SEVERAL ALLOYS

The recommended procedures outlined above were applied to the following aluminum alloys: Aluminum Research Institute (A.R.I.) samples 39 and 40, and Bureau of Standards samples 85 and 86b. The reported complete analyses for these alloys are given in Table I, and a summary of the results obtained for these alloys by the recommended procedures is given in Table II. It is seen from Table II that the agreement between reported values and those found polarographically is satisfactory.

The polarographic curves used in the analysis of Aluminum Research Institute sample 39 are shown in Figures 3 through 8.

Figures 3, 4, and 5 are used to obtain standard values for the various elements determined. The iron wave in Figure 6 was used to determine the percentage of iron. The diffusion current must be corrected for the residual current of aluminum in the absence of iron (curve 1, Figure 3). The concentration of copper in the alloy sample was too large and a smaller volume of alloy solution must be used. This was done in obtaining curve 1 in Figure 7 which was run on 1.000 ml. of alloy solution by the recommended procedure. Lead was determined by the procedure recommended in Section 5 (see curve 2 in Figure 7). Nickel and zinc were determined by the procedure recommended in Section 6 (see Figure 8).

For routine analyses, it will not be necessary to run standard curves for each alloy. Standard curves may be run for the capillary used as the dropping mercury electrode and these curves used in all analyses. In routine work, it would be more convenient to know the ratio of the diffusion current constants for the metals Fe(III), Cu(II), Pb, Ni, and Zn obtained under conditions of the recommended procedures. Thus, if one determines with his own capillary the standard value of one of these metals, the concentration of the other metals can be found from the known ratio of the diffusion currents. Although it is recommended that each worker determine these ratios with his own capillary, the authors are adding the ratios found in their work. The diffusion currents observed for standard samples run according to the recommended procedures and containing 1.00 mg. of metal in 25 ml. of final solution were: for iron, 2.06; for copper, 4.01; for lead, 1.47; for nickel, 3.78; and for zinc, 3.88 microamperes. Thus, for equal amounts of each metal in the solution the ratio referred to ferric iron is: copper 1.95, lead 0.714, nickel 1.83, and zinc 1.88.

The approximate time required for a complete analysis for iron, copper, lead, nickel, and zinc can be estimated roughly as follows:

The preparation of the alloy solution requires about 45 minutes, but solutions of different samples can be made at one time such that the time for each sample will average much less than 45 minutes. If the amounts of iron and copper are not too large, they can be determined in 10 to 15 minutes. Lead can be determined in about 15 minutes and nickel and zinc by the rapid method in about 20 minutes. Thus the total analysis for the five metals can be carried out in about 45 minutes. In routine analyses the actual time spent will be less than 45 minutes, because other manipulations can be carried during the period in which oxygen is removed from the polarographic cells.

The polarographic determination of metals other than those discussed in this paper will be investigated in the future.

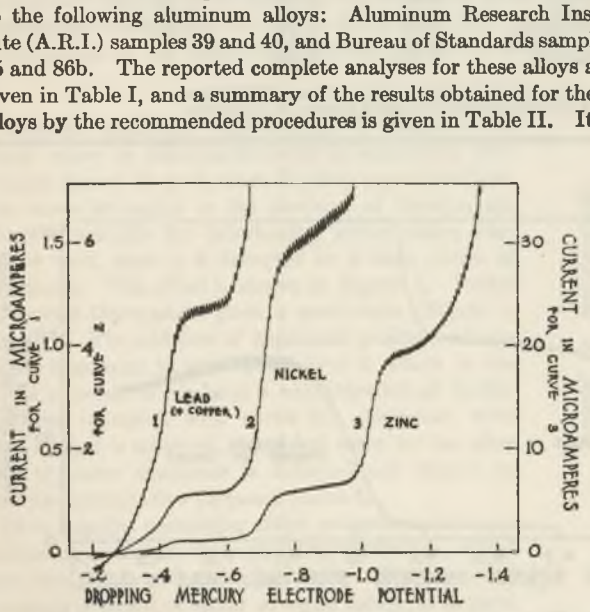

Figure 8. Determination of Nickel and Zinc in Sample A.R.I. 39 (Rapid Method)

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Hume, D. N., and Harris, W. E., IND. ENG. CHEM., ANAL. ED., 15, 465 (1943).
(2) Sandell, E. B., "Colorimetric Determination of Traces of Metals", New York, Interscience Publishers, 1944.

Determination of Petroleum Oil Spray Deposits on Citrus Leaves

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In studying the insecticidal efficiency and phytocidal value of petroleum oil spray deposits on citrus, it has been necessary to determine accurately the amount of oil deposited by oil sprays and the amount retained by the foliage over a period of several weeks. In order to eliminate the error introduced by the extraction of variable amounts of plant waxes and at the same time retain the advantages of gravimetric measurement, it is proposed to sulfonate the extract in petroleum ether, separate the petroleum ether containing the unreacted oil, and weigh the residue after evaporating the ether.

BEFORE the insecticidal efficiency and the phytocidal value of different types of petroleum oils (as their emulsions) for citrus trees can be compared, it is necessary to determine accurately the amount of oil deposited on the foliage by the emulsion. It is also important to be able to determine the amount of oil retained by the foliage over a period of several weeks.

The earlier attempts at estimating oil deposits were based on insect kill and severity of injury to the host plant, while other estimates were based on the difference between the oil contents of the runoff from sprayed surfaces and of the emulsion (*6*, *8*).

English (*7*) described the first method of measuring oil deposits directly, which consisted of extracting with ethyl ether disks cut from sprayed leaves. The solvent was evaporated and the oil plus leaf extract was recovered by centrifuging. Unsprayed leaves were extracted and treated in the same manner to determine the amount of leaf extract. More recent workers (*3*, *5*) have shown that the oil extraction is incomplete and the amount of leaf extract variable. Other workers have used different types of extraction apparatus and have removed the leaf extract by freezing (*4*), or by sulfonating the extract and recovering the unreacted (or raffinate) oil by centrifuging. These methods are limited in accuracy. One of the limitations of the gravimetric method is the variable amount of plant waxes extracted, while the most serious limitation of the volumetric methods is the accuracy in reading the volume of oil recovered.

The only colorimetric method reported (*9*) consists of saturating the oil with an oil-soluble dye, Sudan III, spraying the oil as an aqueous emulsion, and recovering the dye by washing the sprayed object with odorless kerosene. The recovered dye is then compared with color standards containing known amounts of dyed oil. Cressman (*2*) stated that the addition of Sudan III lowered the interfacial tension between kerosene and water from 46 to 25 dynes per cm. However, in the author's experience, addition of Sudan III to three commercially used oils was not observed to lower the interfacial tension more than one dyne in any case, and, moreover, emulsions made in a like manner with dyed and nondyed oils gave comparable oil deposits by the method described below. However, the colorimetric method failed in field experiments, for in practice a small amount of green pigment is extracted from the leaves and the matching with known color standards is difficult. In addition, the color fades more than 50% in less than 2 hours in direct sunlight when known amounts of dyed oil are smeared on citrus foliage.

PROPOSED METHOD

In order to eliminate the error introduced by the extraction of variable amounts of plant extract and at the same time retain the advantage of gravimetric measurement, it is proposed to eliminate the plant extract by treating the petroleum ether extract with sulfuric acid. This causes the plant esters to disappear into the acid layer, leaving an insignificant amount in the ether phase. The petroleum ether containing the unreacted oil is separated and evaporated and the residue is weighed. The amount of oil that reacts with the sulfuric acid can be determined by treating a weighed amount of the same oil in like manner.

REAGENTS. 38 *N* sulfuric acid (*1*) and C.P. petroleum ether (boiling point 30° to 60° C.).

APPARATUS. Standard Soxhlet apparatus, preferably equipped with a stopcock outlet at the bottom of the barrel to permit removal of ether at the end of the refluxing period without dismantling apparatus. Sixty-milliliter separatory funnels. A punch for cutting 1.96-cm. diameter disks.

PROCEDURE. As soon as a spray has dried on the citrus tree, fifty clean mature leaves are picked at random from the outside canopy; 150 disks 1.96 cm. in diameter (3.0 sq. cm. on each surface) are then cut from the leaves and refluxed for 2 hours in a Soxhlet extractor with about 125 ml. of petroleum ether. At the end of the refluxing period, the condensate ether is drawn off the barrel until only about 15 ml. of ether and extract remain in the flask. The apparatus is now dismantled and 10 ml. of 38 *N* sulfuric acid are added to the ether and extract. After 15 minutes at room temperature (20° to 25° C.) with frequent agitation, the petroleum ether is decanted into a 60-ml. separatory funnel. The remaining acid is washed with two 10-ml. portions of petroleum ether and this petroleum ether wash is added to the separatory funnel. (A second separatory funnel is more efficient in effecting separation of the acid and the ether wash.)

The combined petroleum ether fractions are washed with three 10-ml. portions of distilled water. The petroleum ether is then filtered into a tared flask and the separatory funnel and filter paper are washed down with 5 ml. of petroleum ether. The petroleum ether is evaporated on a water bath at 75° by an aspirator (25 to 30 mm. of mercury). The evacuation is continued for one minute after the petroleum ether has apparently all evaporated. The vacuum is released and the flask allowed to come to equilibrium with the atmosphere (40 minutes). The tared flasks are always weighed against a flask to compensate for apparent changes in weight, caused by a difference in temperature and humidity between the initial and final weighings. The difference in weight between the initial and final weighings is the amount of unreacted (raffinate) oil recovered. To determine the proportion of the given mineral oil which will not react under the conditions of this procedure, 50 to 150 mg. of the oil in question are treated in the manner described above. The percentage of oil recovered is designated for the purpose of this method as the raffinate number of the oil, as shown in Table I.

The original amount of oil deposited on the foliage is the amount of oil recovered divided by the raffinate number of the given oil. When the weight of oil deposited is divided by the area of sample taken the oil deposit per unit area is obtained as shown in Table II.

PRECISION AND ACCURACY

When several samples of mature clean leaves were picked from the same area of sprayed citrus trees the average variation in oil deposit is less than ±3 micrograms per sq. cm.

Table I. Determination of Raffinate Number

No.	Sample Weight	Weight Recovered		Raffinate No.
	Mg.	*Mg.*		%
1	54.2	38.9		71.8
2	94.3	68.4		72.5
3	128.3	91.8		71.6
			Av.	72.0 ± 0.3
1	64.8	44.9		69.3
2	82.2	56.2		68.4
3	132.6	90.2		68.0
			Av.	68.6 ± 0.5

When known amounts of oil from 50 to 100 mg. were smeared on citrus foliage, extracted as described above, and corrected for sulfonation the recovery was 100.8 ± 1.6%, shown in Table III.

As a test for the completeness of recovery of the oil, 50 to 100 mg. of white mineral oil (100% unsulfonated residue A.O.A.C.) were smeared on 3 sets of leaves. The recovery was 94.7, 95.0, and 95.1%. However, the refractive index of the oil was lowered from n_D^{25} 1.4692 to n_D^{25} 1.4682, indicating a reaction of the oil with the acid. A sample of oil was treated with 20% fuming sulfuric acid at 75° C. and the unsulfonated oil was separated, washed free of sulfonated material, and then dried. When two sets of leaves were smeared with 50 to 70 mg. of this oil and the leaves were extracted as above, 98.0 and 98.8% of the oil was recovered, but the refractive index was lowered 4 units in the fourth decimal place, which probably indicates a further reaction between the acid and the oil.

To test further for the completeness of extraction after the disks were extracted in the manner described above, the samples were dried, ground, and re-extracted. In no case did the re-extraction give more than 0.3 microgram of oil per sq. cm. of leaf surface. In so far as this method of re-extraction is a valid test (*10*) for the removal of oil, it appears that any oil remaining after the initial 2-hour extraction of the fresh disks is negligible.

INVESTIGATION OF VARIABLES

Rohrbaugh (*10*) has pointed out that the oil is not evenly distributed over the leaf. In the author's experiments when samples were taken from the same area of sprayed trees and disks were cut from the tip of the leaf, the oil deposit averaged from 10 to 15% higher than when random disks were cut. However, the oil deposit on disks cut at random as compared to the oil deposits on whole leaves was within the experimental error of sampling as shown in Table IV. (The area of whole leaves was determined by outlining the leaves on graph paper and computing the area after extraction.) When punches of different sizes were used and extracted with comparable fractions of petroleum ether, the amount of leaf extract increased per unit area the smaller the disks.

The amount of leaf extract from citrus leaves increases with higher boiling petroleum ether, is in the same order of magnitude as the oil deposit, and is variable (Table V). There is more variation between samples than between extractions of different varieties of citrus leaves tested (oranges, grapefruit, and tangerines). When these extracts are sulfonated as described above, there remain from 0.4 to 1.3 mg. (0.4 to 1.4 micrograms per sq. cm.) of material unsulfonated (Table V). Since the amount of leaf extract unsulfonated is small and variable it is neglected and not corrected for in the determination of the true oil deposit.

A slight variation in the concentration of the acid affects materially the sulfonation of the oil, and it has been found expedient for routine laboratory work to include a blank determination in each group of determinations rather than try to keep the concentration of acid at exactly 82.32% SO_3. It has been found that the acid will be diluted from 82.38% to less than 81.88% SO_3 by opening the bottle in the course of using 500 ml. of the 38 *N* acid.

To determine the effect of sulfonation on the petroleum ether, 50 ml. of ether were treated by the 38 *N* acid, and the unaffected ether was separated, washed, and evaporated. The nonvolatile residue was always less than 0.2 mg.

When 55 mg. of a 56 Saybolt second oil (3% distilled at 235° C. and 20% at 264° C.) were dissolved in 50 ml. of petroleum ether (b.p. 30° to 60° C.) and evaporated in the manner described above, no loss in weight could be detected.

When leaves were picked and stored in air-tight containers at 38° F. for periods up to one week, the oil deposit was within the experimental error of sampling.

This method enables the rate of evaporation of petroleum oil from citrus leaves to be followed with a fair degree of accuracy even when the plant extract is many times greater than the oil remaining on the foliage.

Table II. Determination of True Oil Deposit

No.	Oil in Emulsion	Total Area of Sample (Both Surfaces)	Weight of Oil	Raffinate No.	Weight of Oil Deposit	Oil
	%	*Sq. cm.*	*Mg.*	%	*Mg.*	*γ/sq. cm.*
1	1.40	900	52.4	72.0	72.7	80.9
2	1.40	900	52.3	72.0	72.6	80.7
3	1.40	900	51.9	72.0	72.1	80.1
4	1.40	900	53.8	72.0	74.7	83.0
5	1.40	900	52.6	72.0	73.0	81.2
						Av. 81.2 ± 0.8
6	1.75	900	68.1	72.0	94.5	105
7	1.75	900	70.6	72.0	98.1	109
8	1.75	900	68.6	72.0	95.3	106
9	1.75	900	64.0	72.0	88.8	99
10	1.75	900	69.2	72.0	96.2	107
						Av. 105 ± 3
11	1.40	900	50.8	68.6	74.0	82.2
12	1.40	900	50.3	68.6	73.4	81.5
13	1.40	900	49.3	68.6	71.9	79.9
14	1.40	900	50.2	68.6	73.2	81.3
15	1.40	900	52.3	68.6	76.2	84.6
						Av. 81.9 ± 1.2
16	1.00	600	37.9	94.9	39.9	66.5
17	1.00	900	58.0	94.9	61.2	68.0
18	1.00	900	56.6	94.9	59.7	66.3
19	1.00	900	55.8	94.9	58.8	65.4
20	1.00	1200	76.3	94.9	80.4	67.0
						Av. 66.6 ± 0.7

Table III. Recovery of Petroleum Oil from Citrus Leaves

Sample No.	Weight of Sample	Recovery	Raffinate No.	Oil Recovered (Corrected for Sulfonation)	Recovery
	Mg.	*Mg.*	%	*Mg.*	%
1	68.4	49.4	72.0	68.6	100.2
2	96.3	71.2	72.0	98.8	102.5
3	79.8	57.4	72.0	79.7	99.9
4	60.3	40.6	68.6	59.1	98.0
5	86.9	60.4	68.6	88.0	101.2
6	90.3	64.0	68.6	93.2	103.2
				Av.	100.8 ± 1.6

Table IV. Variation of Oil Deposit on Leaf Surface from a 1.4% Oil Emulsion Spray

Sample No.	Total Area, Both Surfaces	Type of Sample	Oil Recovered	Raffinate No.	Oil Deposited	
	Sq. cm.		*Mg.*	%	*Mg.*	*γ/sq. cm.*
1	900	Disk from leaf tip	55.4	72.0	77.0	85.5
2	900		57.2	72.0	79.4	88.3
3	900		58.5	72.0	81.2	90.2
					Av.	88.2 ± 2.3
4	900	Disk at random	49.8	72.0	69.2	76.8
5	900		50.0	72.0	69.5	77.2
6	900		51.4	72.0	71.4	79.4
					Av.	77.8 ± 1.1
7	651	Whole leaves	35.3	72.0	49.1	75.3
8	418		23.3	72.0	32.3	77.3
9	781		46.1	72.0	64.1	82.0
					Av.	78.2 ± 2.5

Table V. Variation of Citrus Leaf Extract from Unsprayed Trees

Sample No.	Boiling Range, Petroleum Ether	Sample Area (6 Sq. Cm. Disks)	Total Leaf Extract		Leaf Extract after Sulfonation	
	° C.	*Sq. cm.*	*Mg.*	*γ/sq. cm.*	*Mg.*	*γ/sq. cm.*
1	33–47	900	14.0	15.6	0.4	0.4
2	33–47	900	17.3	19.2	0.6	0.7
3	33–47	900	26.1	29.0	0.9	1.0
			Av.	21.3 ± 5.2		0.7 ± 0.2
4	43–74	900	46.6	51.8	1.3	1.4
5	43–74	900	23.9	26.6	0.7	0.8
6	43–74	900	38.9	43.2	1.0	1.1
			Av.	40.5 ± 9.2		1.1 ± 0.2

The sample size as well as the disk size taken here is for convenience and other sizes work equally well. Precision of the method seems to be in proportion to number of leaves sampled.

LITERATURE CITED

(1) Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis, 5th ed., 1940.
(2) Cressman, *J. Econ. Entomol.*, 34, 804 (1941).
(3) Dawsey, *J. Agr. Research*, 52, 681 (1936).
(4) Dawsey and Hass, *Ibid.*, 46, 41 (1933).
(5) Dawsey and Hiley, *Ibid.*, 55, 693 (1937).
(6) DeOng, Knight, and Chamberlin, *Hilgardia*, 2, 351 (1927).
(7) English, *J. Agr. Research*, 41, 131 (1930).
(8) Griffin, Richardson, and Burdette, *Ibid.*, 34, 727 (1927).
(9) McCall and Kagy, *J. Econ. Entomol.*, 33, 905 (1940).
(10) Rohrbaugh, *Plant Physiol.*, 9, 699 (1934).

PRESENTED before the Florida Section of the AMERICAN CHEMICAL SOCIETY, Tampa, Fla., May 12, 1945, and in the Meeting-in-Print, AMERICAN CHEMICAL SOCIETY, September, 1945.

Estimation of Sucrose and Lactose in Binary Mixtures

With Particular Application to Sweetened Condensed Milk

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THE method described here for estimating sucrose and lactose is based on the fact that sodium bisulfite decreases the optical rotation of aldose sugars (*3*), and that the optical rotation of a mixture of two sugars, at least one of which is such a sugar, can be made a linear function of the concentration of each sugar. A method for the determination of maltose and dextrose in a binary mixture has been reported earlier (*2*). The method herein described consists of two steps: the determination of the percentage of total sugars by means of the refractometer and the measurement of the optical rotation of a solution containing a known percentage of total sugars in the presence of sodium bisulfite.

For the actual determination of these sugars, a curve is first plotted using the data in Table I, the rotation being measured in a Schmidt and Haensch saccharimeter.

The data in Table I were obtained by dissolving the sugars in the ratios given in sugar dilution flasks, adding 30 grams of sodium metabisulfite per 10 grams of sugar, making up to the 110-ml. mark with distilled water, letting stand (stoppered) about 2 hours at 20° C., and then reading the rotation.

It was found that when the rotations corresponding to the two 100% points were plotted, and then connected by a straight line, the intermediate points all lay practically on this line, the sucrose-lactose rotation plot shown in Figure 1.

METHOD

In applying the method to sweetened condensed milk, the most commonly occurring mixture of these two sugars, dissolve a known weight of the sample, add a portion of 5% copper sulfate solution (3 ml. per 10 grams are suggested) to clarify; and make up to a volume numerically equal to twice the weight of sample with distilled water. A concentration greater than 50% is difficult to mix thoroughly and filter; a lesser concentration yields inaccurate results. Filter, using a vacuum if necessary. The filtrate will have a pH of about 4.5. Then measure the index of refraction of the filtrate. Since only a drop is necessary the sample need not be large. Convert the index of refraction to sucrose (*1*), which can be used without great error for other sugars, and hence can be used for total sugars.

Compute the size of sample required to give 10 grams of total sugars, then weigh out, dissolve in water, clarify, and make to a volume of 100 ml. in a sugar flask. Now treat 100 ml. of clarified sample with 30 grams of sodium metabisulfite and make to dilution mark with distilled water. After 2 hours filter on a pressure filter (not vacuum), conveniently made with a fritted-glass filter (about 50 mm. which will hold about 50 ml.) which will retain the matter suspended in the solution (possibly sulfur). Polarize and take off the corresponding percentage sucrose (or percentage lactose) from the plot in Figure 1. This multiplied by the "per cent total sugars" will give the per cent sucrose (or lactose) in the sample of condensed milk.

Table I. Optical Rotation of Sucrose-Lactose (Hydrate) Mixture

(10 grams in 30% bisulfite solution at 20° C. with 200-mm. tube)

Sucrose, %	0	20	40	50	60	80	100[a]
Lactose, %	100	80	60	50	40	20	0
S°[b]	8.2	13.1	18.4	21.0	23.2	28.3	33.9[a]

[a] This is a good test for purity of the sucrose; a commercial granulated sugar gave a rotation of 32.9.
[b] Degrees on international sugar scale.

Table II. Lactose and Sucrose in a Sample of Sweetened Condensed Milk

	Total Sugars	Lactose/ Sucrose Ratio	Lactose	Sucrose
	%		%	%
Composition (manufacturer's)	55.62	23.1/76.9	12.87[a]	42.75
Author's analysis	56.0	24/76	13.4	42.6

[a] Calculated as 0.545 × milk-solids-not-fat.

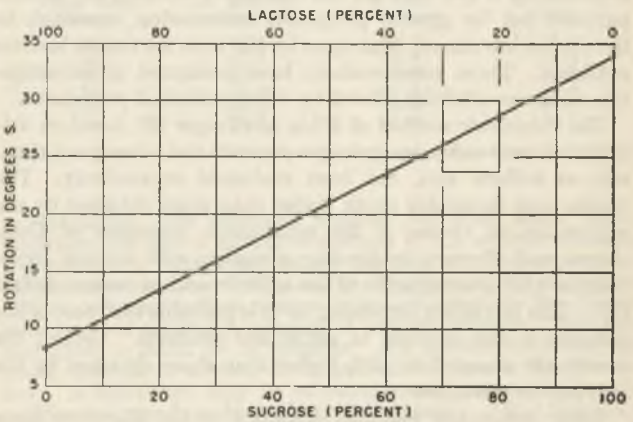

Figure 1. Sucrose-Lactose Rotation

In Figure 1 is shown a small inset, covering all sucrose-lactose concentrations in sweetened condensed milk with the corresponding rotations, so that if an enlarged plot is made with these limits, and the plotted section of the locus drawn on it at a steeper angle, a plainer intersection can be made with the rotation with a corresponding increase in accuracy.

LITERATURE CITED

(1) Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis, 5th ed., p. 667, 1940.
(2) Browne, H. H., IND. ENG. CHEM., ANAL. ED., 16, 582 (1944).
(3) Browne, H. H., *J. Org. Chem.*, 9, 477 (1944).

Determination of Total Sulfur and of the Gamma Number of Viscose

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A procedure is described for the rapid volumetric determination of carbon disulfide combined in viscose. The sulfur in all forms, whether combined as sodium cellulose xanthate, sodium thiocarbonate, or sodium sulfide, is quantitatively transformed into zinc sulfide in the presence of sodium zincate. The zinc sulfide is then titrated iodometrically in the presence of an excess of sulfuric acid. The method is modified to determine the viscose gamma number by titrating the by-product sulfur separately after salting out the sodium cellulose xanthate.

IN GENERAL, the industrial routine control of viscose is limited to the determination of total alkalinity, cellulose content, ripeness index, and viscosity. Frequently, however, the determination of total sulfur as carbon disulfide is a desirable addition to this group of analyses—e.g., in order to control the uniformity of xanthation, to check the efficiency of carbon disulfide manipulation, or to locate leaky reactors.

DETERMINATION OF TOTAL SULFUR

The only reliable method known hitherto for the determination of total combined carbon disulfide consists in transforming the total sulfur present into sulfuric acid through an oxidation process and determining it quantitatively as barium sulfate. The procedure requires skill, space, and considerable time. For these reasons, in practice only occasional determinations are made by it and they are not conducted routinely for control purposes but for general production information, inasmuch as the viscose has already been spun by the time the results become available. These considerations have prompted an investigation of the possibilities offered by other published procedures.

The volumetric method of d'Ans and Jaeger (*3*), based on oxidation of total sulfur by hydrogen peroxide and subsequent titration as sulfuric acid, has been evaluated exhaustively. The results were invariably much higher than those obtained by the application to viscose of the gravimetric procedure of Delachanal and Mermet, embracing oxidation with sodium hypobromite and determination of the sulfuric acid as barium sulfate (*4*). This fact is not surprising, for it is probable that part of the cellulose is also oxidized to acidic end products. Indeed, the results are always 7 to 10% higher than those obtained by the gravimetric procedure.

Other volumetric methods described in the literature have generally been applied to sulfur combined only as cellulose xanthate, which does not indicate the extent to which the allotted carbon disulfide has been utilized. Then, too, many contradictory findings have been reported. The method of Cross and Bevan (*2*), for instance, which is an adaptation of a reaction discovered by Delachanal and Mermet (*5*), gives results which do not always agree with the gravimetric determination of sulfur in cellulose xanthate. The probable explanation is that the transformation of the sodium cellulose xanthate into a dixanthylated derivative in the presence of iodine is not a complete reaction, as was brought out by André (*1*) while working on potassium ethyl xanthate. A complete survey of the literature on analytical methods for viscose is given by Ott (*10*). Changes involved in the ripening of viscose, are discussed by Moore (*9*), Eckert and Hauan (*6*), and Fink (*7*).

For a correct understanding of the problem, it is necessary to keep in mind that sulfur is present in viscose in three chemical combinations: the cellulose xanthate complex, $R_{Cel.}CS_2Na$, and two end products of its ripening, sodium sulfide, Na_2S, and sodium thiocarbonate, Na_2CS_3. The last compound is stable only in concentrated solution or in solid form (*11*). In dilute solution it hydrolyzes more or less rapidly according to the reaction:

$$Na_2CS_3 + 3H_2O = Na_2CO_3 + 3H_2S$$

The possibility was first investigated of utilizing this reaction to determine thiocarbonates as sulfides. The rate of the reaction is considerably accelerated upon substituting the metal of an amphoteric oxide for sodium. The experimental data have proved that, in the presence of sodium zincate, the reaction is carried to completion after 15 to 20 minutes' boiling time, provided the dilution is sufficient and the alkalinity not too high:

$$Na_2CS_3 + 3Na_2ZnO_2 + 3H_2O = 3ZnS + Na_2CO_3 + 6NaOH$$

and that zinc sulfide can be readily titrated iodometrically in the presence of an excess of a strong acid.

The next step was to ascertain if a similar reaction would be given by sodium alkyl xanthates. Solutions of sodium ethyl xanthate were demonstrated to be hydrolyzed quantitatively after 15 to 20 minutes' boiling time, according to the reaction:

$$C_2H_5OCS_2Na + 2Na_2ZnO_2 + 2H_2O = 2ZnS + C_2H_5OH + Na_2CO_3 + 3NaOH$$

Sodium plumbite reacted similarly. It was discarded, however, because of the difficulty of titrating lead sulfide iodometrically after completion of the reaction.

When it was thus learned that the sulfur from mixtures of sodium ethyl xanthate and sodium thiocarbonate could be transformed quantitatively into zinc sulfide, the following procedure was worked out in order to convert the total sulfur in viscose into zinc sulfide for its subsequent iodometric titration.

REAGENTS. A sodium zincate reagent composed of 10 grams of C.P. zinc oxide, 100 grams of C.P. caustic soda, and distilled water to 1000 ml.; 0.1 *N* iodine, 0.1 *N* thiosulfate, and *M* sulfuric acid solutions.

PROCEDURE. Into a cold 150-ml. beaker weigh exactly 25 grams of viscose to the centigram. Transfer it to a 250-ml. volumetric flask with cold distilled water and complete the volume. Into a 500-ml. Erlenmeyer flask introduce in the order given 100 ml. of distilled water, 10 ml. of sodium zincate reagent, and a 10-ml. aliquot of the diluted viscose. Cover with a 5-cm. diameter funnel to avoid spattering. No immediate precipitation occurs. The mixture clouds between 65° and 70° C. and flocculation starts at 75° C. (A yellow precipitate appears overnight if the solution is kept at room temperature.)

Bring the contents to a boil. After 30 minutes of gentle boiling, cool the contents of the flask in ice, then flush into a 600-ml. beaker containing a cold solution of 50 ml. of *M* sulfuric acid and 20 ml. of 0.1 *N* iodine. Immediately transfer the mixture back into the Erlenmeyer flask quantitatively. Allow a minimum of 20 minutes of contact to decompose the zinc sulfide entirely, then titrate the excess iodine not consumed with a 0.1 *N*

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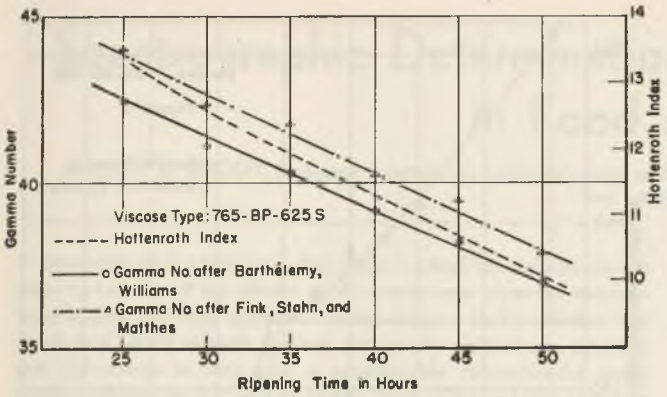


Figure 1

solution of sodium thiosulfate in the presence of a few drops of starch solution as indicator. The sulfur content as CS_2 is obtained from the equation:

$$\% CS_2 = 0.1903 \times \text{ml. of } 0.1\ N \text{ iodine consumed}$$

RESULTS. A sample of viscose analyzed ten times according to this procedure gave the following results:

2.353	2.344	2.353	2.344	2.344
2.344	2.353	2.363	2.335	2.353

Arithmetic mean: 2.349% CS_2.
Standard deviation for a single determination: 0.007% CS_2.

Two determinations carried out on the same sample according to the gravimetric method of Delachanal and Mermet (oxidation by sodium hypobromite) afforded 2.340 and 2.338%.

Another viscose sample compounded with slightly more carbon disulfide gave the following results:

2.363	2.358	2.363	2.355	2.351
2.358	2.352	2.360	2.353	2.358

Arithmetic mean: 2.357% CS_2.

Two check determinations, carried out according to the conventional gravimetric method already mentioned, afforded 2.365 and 2.360, respectively.

Not only are the results obtained with each of these two methods in good agreement, but when speed is an important factor, as is always the case in a plant operation control laboratory, this new volumetric procedure has a decided advantage over the conventional one. The time required by a technician to run a total carbon disulfide combined is approximately as follows:

Volumetric method: from 1 hour 40 minutes to 2 hours
Gravimetric method: from 16 hours to 20 hours

DISCUSSION. The preparation of the sample by taking an aliquot from 25 grams of viscose seems unnecessarily cumbersome in contrast with the direct weighing of 1 gram. Experience, however, shows that the longer procedure invariably yields better duplication, perhaps because it minimizes surface evaporation.

Adding the acidified iodine solution to the products of the sodium zincate reaction, instead of flushing the products into the iodine solution as recommended, gives high results, because some iodine then reacts with caustic soda before complete neutralization takes place. Acidifying the products of the reaction before adding the iodine solution, and then titrating, is also an undesirable procedure, for some hydrogen sulfide always escapes, even under strong cooling.

The quantitative transfer of products and acidified iodine solution back into the original Erlenmeyer flask is also essential. Often very small amounts of zinc sulfide adhere to the flask and are difficult to remove quantitatively. The small opening of the flask also lessens the loss of iodine vapor.

The minimum of 20 minutes of contact allowed to decompose the zinc sulfide by the excess iodine solution has been established experimentally. Warming would accelerate this phase of the operation, but, if accurate results are desired, warming is to be avoided on account of the volatility of the iodine.

In addition, the precautions usual in iodometric titrations must be taken. It is not always appreciated that the vapor pressure of iodine over 0.1 N iodine–potassium iodide solution is of the order of 0.3 mm. of mercury, equivalent to about 4 mg. of iodine per liter of air in equilibrium with the solution. For this reason it is necessary to precool the solutions, to cover the flask in which the decomposition of the zinc sulfide is performed, and to avoid air drafts if analyses are to be duplicated closely.

GAMMA NUMBER

The gamma number of a viscose is an important characteristic, defined as the number of xanthate groups per 100 anhydro-glucose units of the cellulose. In the original method of Fink, Stahn, and Matthes (*8*), the cellulose xanthate is precipitated by reaction with diethylchloroacetamide. The nitrogen content of the precipitate is found by the Kjeldahl method, and the gamma number is calculated from the equivalence between nitrogen and xanthate sulfur.

The method set forth above may be extended readily to obtain gamma numbers. If the sodium cellulose xanthate is first precipitated by salting out and is filtered from the solution, it is assumed that the filtrate will contain only by-product sulfur—i.e., sodium thiocarbonate and sodium sulfide, which can be determined by the same procedure used for total sulfur. The difference between total and by-product sulfur is xanthate sulfur, from which the gamma number is calculated.

Table I. Gamma Number of Commercial Viscose

Aging Time, (Hours)	Gamma Number B. and W. procedure	Gamma Number F.S.M. procedure	Hottenroth Index
25	42.5	44.1	13.4
30	41.1	42.4	12.6
35	40.3	41.7	12.0
40	39.1	40.2	11.4
45	38.1	39.5	10.7
50	36.8	37.9	10.1

REAGENTS. The solutions required for total sulfur determination and also a saturated solution of C.P. sodium chloride.

PROCEDURE. Pipet 100 ml. of the diluted viscose prepared for the determination of total sulfur (25 grams in 250 ml.) into a 500-ml. volumetric flask packed in ice and complete the volume with an iced saturated solution of sodium chloride. Mix well and allow to stand for 15 minutes. (In the case of unripe viscoses, the rate of settling of coagulated xanthate is likely to be low. A centrifuge may be advantageously used to hurry the settling and speed up the analytical procedure.) Filter through a dry fluted paper of rapid filtering characteristics. Pipet 50 ml. of the filtrate into a 500-ml. Erlenmeyer flask and determine its sulfur content as carbon disulfide by the method described. Calculate the gamma number from the equation:

$$\text{Gamma No.} = \frac{213\ (\%\ \text{total } CS_2 - \%\ \text{by-product } CS_2)}{\%\ \text{cellulose}}$$

RESULTS. In Table I are shown the results obtained by this method for the gamma number of a sample of commercial viscose. The results by the original method of Fink, Stahn, and Matthes and for the Hottenroth index are also given for the same sample at various degrees of ripeness. The data are presented graphically in Figure 1.

In Table II are shown the results obtained by determining the

Table II. Determination of Carbon Disulfide

Hottenroth Index	CS_2 Combined as Sodium Cellulose Xanthate: B. and W. procedure	Gravimetric procedure	Difference
	%	%	%
15.0	1.514	1.510	−0.004
14.0	1.463	1.470	+0.007
13.0	1.412	1.410	−0.002
12.5	1.391	1.390	−0.001
12.0	1.377	1.383	+0.006
11.5	1.352	1.358	+0.006
11.2	1.347	1.348	+0.001
11.0	1.341	1.346	+0.005
10.8	1.341	1.345	+0.004
10.6	1.308	1.310	+0.002
10.4	1.270	1.280	+0.010
9.9	1.223	1.228	+0.005
9.0	1.129	1.135	+0.006
8.4	1.069	1.072	+0.003

carbon disulfide combined as sodium cellulose xanthate in viscose of different indexes:

(% total CS_2 combined) − (% CS_2 combined as by-products)

In the third column are the results obtained by carrying out the same determinations according to the conventional gravimetric method. These data are presented graphically in Figure 2 as a function of the Hottenroth index.

The ripening curve is characteristic, for it presents a sharp discontinuity for a Hottenroth index of 10.8. This discontinuity is not detected when the index is expressed as a function of the time of ripening. For this reason it escaped the attention of former chemists who devoted their attention to the ripening of the viscose.

The shape of this ripening curve is a true characteristic of a given viscose. It depends on the chemical composition of the viscose and has proved to be of great technical value.

Discussion. From the standpoint of manipulation it should be remembered that dilution accelerates considerably the rate of ripening of viscose; consequently thorough cooling of all solutions is necessary to obtain consistent results.

Table I demonstrates that the gamma number as secured by this method is consistently lower than the value obtained by the method of Fink, Stahn, and Matthes. After precipitation of sodium cellulose xanthate by diethylchloroacetamide, Fink, Stahn, and Matthes allow a relatively limited amount of water for washing. It is possible that their precipitate retains adsorbed diethylchloroacetamide and that in the method introduced here the precipitate retains by-product sulfur. The cause of the discrepancy is a question open for further investigation.

It is believed that the procedure offered in this paper is shorter than the diethylchloroacetamide method. Moreover, the preparation and manipulation of diethylchloroacetamide, an expensive and hazardous chemical, are evaded.

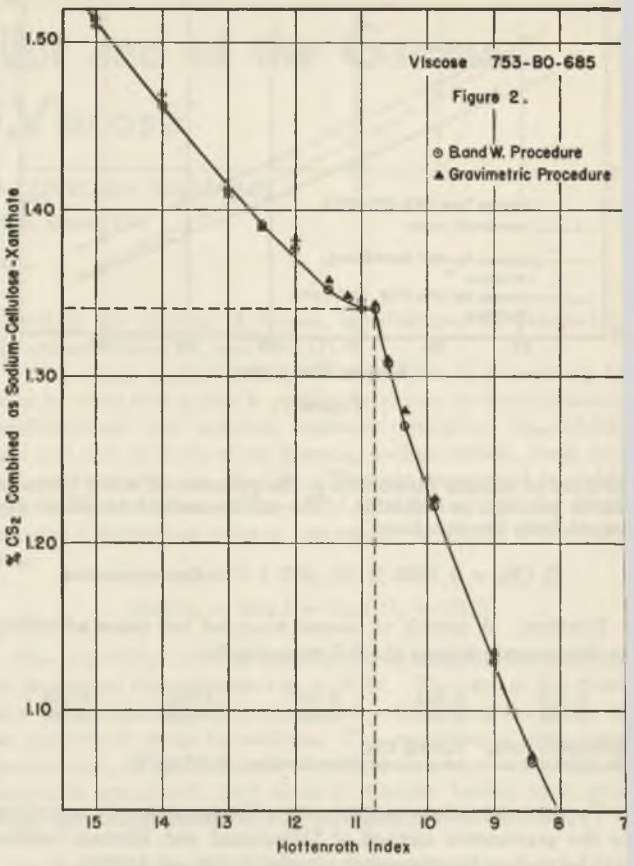
CONCLUSIONS

The new volumetric procedure presented in this paper is satisfactory for determining the total carbon disulfide combined in viscose. It is simpler and much faster than the conventional gravimetric procedure.

It is particularly well suited for prompt location of any leaky carbon disulfide reactor and therefore can contribute efficiently to better plant control operation. Failure to appraise accurately and at the proper time any deviation from standard is often the cause of misdirected effort, deterioration of quality, and unnecessary expense.

Reproducibility and degree of accuracy compare well with the conventional gravimetric method currently used in viscose plants. The accuracy falls within permissible limits of variation.

In determination of the gamma number or of xanthate sulfur,



the suggested procedure is obviously handicapped by the possibility of incompletely salting out the sodium cellulose xanthate, or by the possibility that the precipitated sodium cellulose xanthate may retain by-product sulfur. Nevertheless, where great accuracy is not required, this volumetric procedure makes possible the running of a set of determinations with ease and rapidity.

Another advantage over the conventional gravimetric method is the fact that sulfates, if present, do not react, and consequently do not interfere with the result.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) André, *Bull. soc. chim.*, 33, 1678 (1923).
(2) Cross and Bevan, *Ber.*, 34, 1513–17 (1901).
(3) D'Ans, J., and Jaeger, A., *Cellulosechem.*, 16, 22–36 (1935).
(4) Delachanal and Mermet, *Ann. chim. phys.* (5), 9, 572, 1288 (1874–83).
(5) Delachanal and Mermet, *Compt. rend.*, 81, 92 (1875).
(6) Eckert, P., and Hanan, R., *Klepzig's Textil.-Z.*, 41, Heft 15, 205–13 (1938).
(7) Fink, H., *Z. angew. Chem.*, 47, 429 (1934).
(8) Fink, Stahn, and Matthes, *Ibid.*, 47, 602–7 (1934).
(9) Moore, C. L., *Silk and Rayon*, 8, 563–83 (1934).
(10) Ott, Emil, ed., "Cellulose and Cellulose Derivatives", pp. 834–7, New York, Interscience Publishers, 1943.
(11) Pascal and Baud, "Traité de Chimie Minérale", Vol. V, pp. 233–4, Paris, Masson & Cie., 1932.

Spectrographic Determination of Some Metallic Elements in Food and Feces

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A direct current arc method is described in which special attention is given to a study of the effects of the extraneous elements encountered in food and fecal samples. In the study, a buffer solution was developed which permits sodium, potassium, magnesium, calcium, and phosphorus to vary over a considerable concentration range without influencing the ratio of certain lines of the metallic elements to an internal standard bismuth line. When this buffer solution is used, good comparisons between spectrographic and chemical results are obtained. Certain other advantages pertaining to the development of working curves and to corrections for background are attributed to the buffer solution.

DEVELOPMENT of analytical methods which provide accurate results with biological materials is always hampered by the complexity of substances to be examined and by the wide range of concentration among the many components. Spectrographic methods for the determination of metallic elements in biological material have improved rapidly in recent years, coincident with improvements in spectrographic equipment, rapid development of spectrographic techniques, and the purity and availability of standard materials, as well as an increased awareness of fundamental problems such as the effect of extraneous elements and background corrections.

The concentrations of many of the heavy metals in biological samples fall in a range well suited to spectrographic analysis. Consequently, numerous authors have used the spectrograph for this type of work (*2*, *3*, *4*, *7*, *11*, *12*, *13*, *17*). Of the more recent investigators, Cholak and Story (*2*, *3*, *4*) have made intensive investigations of metals in biological materials. As part of a comprehensive study of mineral metabolism in childhood (*14*) the authors have evolved modifications of the spectrographic methods of Cholak for use in the analysis of food and feces for iron, manganese, copper, lead, tin, and aluminum. From a study of the effects of variable amounts of the extraneous elements, sodium, potassium, calcium, and magnesium, they have developed a spectrographic buffer solution which permits these elements to vary over large ranges of concentration without affecting the ratios of the trace element line to an internal standard line. These effects have been eliminated, primarily by a method of excess which was used by Cholak (*2*) with the direct current arc and by Duffendack *et al.* (*5*), employing an uncondensed spark with solutions containing cadmium as the internal standard element.

The authors, using bismuth as an internal standard and a direct current arc, have determined the actual concentration of sodium and potassium necessary to eliminate the effects of all the extraneous elements found in food and fecal samples. The samples analyzed were obtained during a metabolic balance study of eight children during eleven consecutive 5-day periods. Each sample represented a composite for 5 days. The method of compositing and drying the food and feces samples has been described (*14*). Aliquots of the sample material were analyzed by chemical methods following collection, and the remaining material was kept in airtight glass containers pending later spectrographic analysis. Data for each child for iron, manganese, copper, aluminum, lead, and tin are included in the published data from the study (*15*).

EQUIPMENT

The equipment used included a Bausch & Lomb medium quartz spectrograph (Bausch & Lomb Optical Co., Rochester, N. Y.), supplemented by a Dietert direct current arc rectifier unit (Harry W. Dietert Co., Detroit, Mich.), Leeds & Northrup Micromax recording spectrophotometer (Leeds & Northrup Co., Philadelphia, Pa.), and Dietert mechanical developing machine, dryer, and calculating board. The solutions were handled with a Dietert micropipet and the spectrograms obtained on Eastman spectrum analysis plates, Nos. 1 and 2 (Eastman Kodak Co., Rochester, N. Y.).

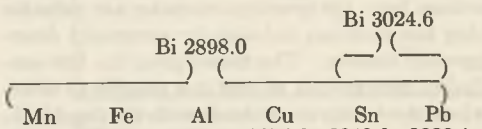
PROCEDURE

Ten-gram samples of the dry food composites were weighed into platinum crucibles and ashed in a muffle furnace in which the temperature was gradually increased to 500° C. and maintained for 6 hours. The ash was allowed to cool, then dissolved in 10 ml. of a buffer solution containing bismuth as an internal standard. The complete development of the buffer solution is described below.

Sufficient amounts of dry feces to provide in excess of 0.25 gram of ash were weighed into platinum crucibles and ashed by the foregoing procedure, allowed to cool, and weighed. In a 25-ml. volumetric flask, 0.25 gram of the ash was made to volume with the buffer solution.

With the micropipet, 0.1 ml. of ash solution was expelled onto the center post of the carbon electrode and allowed to dry for one hour at 120° C. [Acheson grade carbon electrodes were used after rigid purification by the method of Staud and Ruehle (*18*). The carbons were cut to form the crater-centerpost type electrode, pre-arced in a direct current arc at 10 amperes, 50 volts for 7 seconds. Pre-arcing causes the center post to become very porous, so that most of the solution is absorbed. The carbons were placed in an oven thermostatically maintained at 120° C.] After evaporation was complete the carbons were arced for 30 seconds at 7 amperes, 30 to 35 volts, with the cut-out sector set to allow 37% of the incident light to enter the slit (35 microns). For the analysis for tin and lead, the same ash solution was used but the optical intensity was increased by using a 20-second exposure and allowing 100% of the light to fall on the slit. The spectra were recorded on Eastman S.A. No. 1 plates (S.A. No. 2 plates proved more satisfactory for lines of very low intensity) and the plates developed at 18° C. for 3 minutes in Eastman D-19 developer, with mechanical agitation.

The lines used in the analyses are:



Emulsion Calibration Curve by Rotating Seven-Step Sector
Cu — 2998.4 Cu — 3073.8

The manganese, iron, aluminum, and copper lines were all compared to the internal standard line of bismuth (2898.0 Å.) and the analyses for all four elements conducted simultaneously. The tin and lead lines were compared to bismuth line 3024.9.

In the use of the spectrograph for quantitative determinations certain possibilities of interfering lines must always be considered. The microphotometer traces show that the aluminum line 3082.2 Å. is separated from the iron line 3083.7 Å. and allows an accurate determination of the former element. Certain lines of cobalt and vanadium might partially mask the aluminum line 3082.2 Å., if the former elements are present in sufficient quantities. However, the question of interference by the lines of cobalt and vanadium was eliminated by the occurrence of only very small amounts, if any, of these elements in food and by the absence of much stronger lines of the same elements under the exposure conditions used.

In considering the interference of iron lines with the tin and

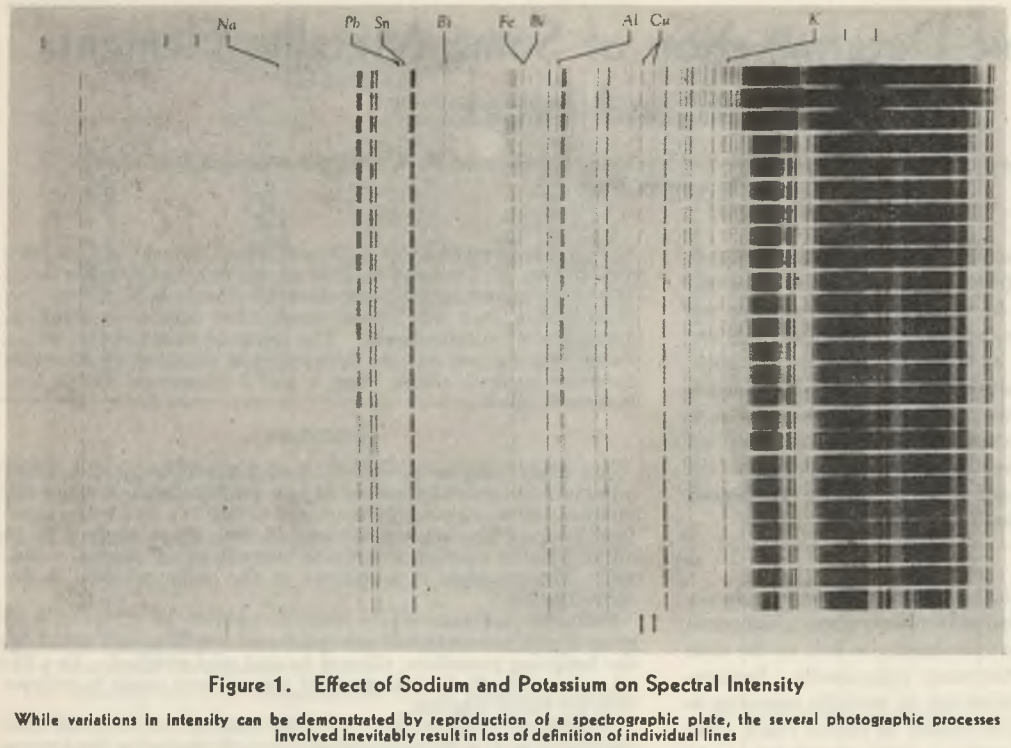


Figure 1. Effect of Sodium and Potassium on Spectral Intensity

While variations in intensity can be demonstrated by reproduction of a spectrographic plate, the several photographic processes involved inevitably result in loss of definition of individual lines

lead lines employed, a similar reasoning was followed. When iron lines of equal or greater intensity than the interfering iron lines were found to be absent from the spectra after visual and microphotometric observations, the question of interference by iron lines of lesser intensity was eliminated or at least held to some point well below the threshold value of the plate. The buffer solution developed in the investigation suppresses the iron lines to such an extent that the interference is eliminated. The intensities given by Harrison (*8*) were used in considering interference.

The line densities were measured with the recording spectrophotometer. The clear plate reading, I_0, was set at zero and total blackness at infinity. Since the scale was logarithmic, the deflection for a given line represented density units, $\log_{10} I_0/I$. (The inked recordings from the spectrophotometer are valuable not only for giving line densities but also for accurately determining the background density. The record gives the line contour with the adjacent background, so that it is possible to determine precisely where the background blends with the line blackening. This gives the recording instrument some advantage over the nonrecording type.)

The emulsion calibration curve was obtained by exposing a copper arc through a rotating seven-step sector wheel. The Hurter and Driffield curve (*10*) was plotted (density *vs.* logarithm of relative exposure). Working curves were prepared by running standard solutions under the conditions to be used with the ash solution. These curves were plotted, $\log_{10}$ concentration *vs.* $\log_{10} \frac{I \text{ element}}{I \text{ bismuth}}$, where I represents the relative intensity.

Corrections for residual amounts of iron, aluminum, and copper were accomplished by a series of approximations, estimating the separation of the curved portion of the working curve from the straight-line portion drawn through the points of higher concentration, as described by Pierce and Nachtrieb (*16*).

Only slight background corrections for copper were necessary, and all other lines were free from background. Since this density was very small compared to line density and the background correction was of the same magnitude for standard and unknown alike, the correction for copper was made by subtracting the background density from the line density.

EFFECT OF EXTRANEOUS ELEMENTS

In the preliminary work on food and feces samples the general over-all intensity of the spectra was decreased by the increased amounts of sodium and potassium, as was observed by Brode (*1*). Standard solutions containing known amounts of the elements to be studied and varied amounts of sodium and potassium were exposed in triplicate. Figure 1 shows the effect within the range from 20 mg. of sodium and 8 mg. of potassium per ml. to 2 mg. of sodium and 1.6 mg. of potassium per ml. The spectral ratio of the lines of certain metallic elements to those of the internal standard, bismuth, increased with increased amounts of sodium and potassium, within certain concentration ranges of these two elements. It was apparent that some concentration range of these elements must be found within which these spectral ratios would remain unchanged for varying amounts of sodium and potassium.

This effect was studied by making additional synthetic standard solutions in which the amounts of sodium and potassium varied but the amounts of calcium, magnesium, and the metallic elements were held constant.

Solutions A and B were identical except that to standard B were added 20 grams of sodium and 16 grams of potassium per liter, as their chlorides. Solution B was diluted with solution A to provide solutions of intermediate concentrations. These solutions were analyzed by the procedure used with the food and feces ash solutions. The relative intensities of the element lines and the internal standard bismuth line were plotted against the concentrations of sodium and potassium. The curves for manganese, iron, copper, and aluminum are shown in Figures 2 and 3. The ratio of an element line to the internal standard bismuth line for a given standard was calculated by dividing the relative intensity of the element line by the relative intensity of the bismuth line. These ratios are shown by the broken-line curves in Figures 2 and 3.

The curve for manganese-bismuth becomes constant with concentrations of 8 mg. of sodium and 6 mg. of potassium per ml., or greater, which indicates that the amounts of sodium and potassium may vary within the range above these concentrations without affecting the spectral ratio (Figure 2). However, the iron-bismuth ratio does not become constant at 8 mg. of sodium per ml. In Figure 2 a slight maximum is shown, representing a deviation of 0.001 mg. per ml. in a concentration of 0.025 mg. per ml., a variation well within the error of the method. With copper (Figure 3), increasing amounts of sodium and potassium had less effect on the copper-bismuth ratio than on the ratios for manganese and iron. In the curve for aluminum, the constantly increasing aluminum-bismuth ratio suggests the possibility of aluminum contamination in the sodium and potassium salts, although qualitative examinations did not reveal any of the element. However, considering the probable error of the method, the variation in the aluminum-bismuth ratio is of little significance.

Table I. Effect of Calcium on Spectral Line Ratio

Calcium Known	Manganese Present	Manganese Found	Iron Present	Iron Found	Aluminum Present	Aluminum Found	Copper Present	Copper Found
	Milligrams per milliliter							
0	0.0064	0.0065	0.021	0.025	0.014	0.013	0.0058	0.0060
1	0.0064	0.0064	0.021	0.022	0.014	0.015	0.0058	0.0054
2	0.0064	0.0062	0.021	0.023	0.014	0.014	0.0058	0.0055
3	0.0064	0.0060	0.021	0.022	0.014	0.016	0.0058	0.0054
4	0.0064	0.0064	0.021	0.022	0.014	0.016	0.0058	0.0050

Although no curves for tin and lead are presented, these elements also were studied. The relative intensity of the tin and lead lines, 2840.0 and 2833.1 Å., respectively, were decreased by increasing amounts of sodium and potassium. However, the ratios of lead and of tin to bismuth remained very constant. In considering the position of these three elements in the periodic table, we might expect their atomic and physical characteristics, and therefore their volatility in the arc, to be similar.

To determine any effect of variable amounts of calcium, a group of synthetic biological solutions was analyzed, in which calcium was made to vary while all other constituents were held constant. Concentrations of calcium varying between 0.0 and 4.0 mg. per ml. in solutions in which the concentrations of sodium and potassium were greater than 8 and 6 mg. per ml., respectively, did not cause variation in the ratios for manganese, iron, copper, or aluminum to bismuth. Since the concentration of these elements is dependent upon their ratios the effect is demonstrated by the values in Table I.

SPECTROGRAPHIC BUFFER SOLUTION

To determine these metals in food and feces spectrographic buffer solutions were developed which eliminate interference by extraneous elements (Table II). The buffer for food ash contains less sodium and potassium than that for fecal ash, since food contains variable and appreciable amounts of these elements. The buffers were designed to throw the concentration of sodium and potassium between 8 mg. of sodium and 6.4 mg. of potassium per ml. and 20 mg. of sodium and 16 mg. of potassium per ml.

Table II. Spectrographic Buffer Solutions

	Composition of Solutions for Dissolving Ash	
	Feces	Food
Bismuth, gram	0.450	0.450
Ammonium chloride, grams	18.0	18.0
Sodium chloride, grams	30.48	12.70
Potassium chloride, grams	17.29	7.52
Hydrochloric acid, high purity, ml.	250	250
Nitric acid, high purity, ml.	250	250
Water, double-distilled, to make 1 liter of solution		

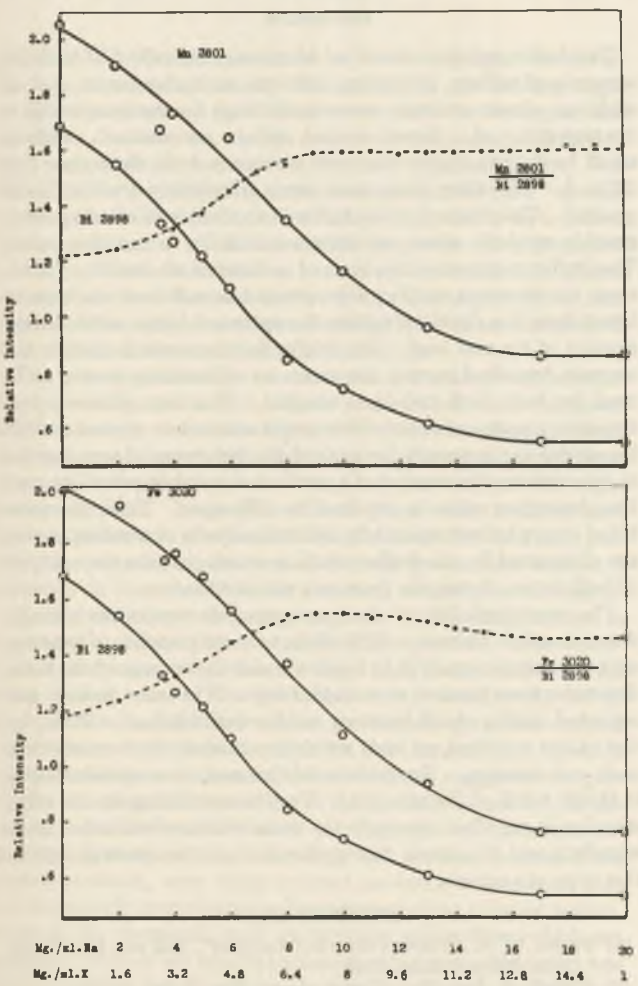


Figure 2. Effect of Sodium and Potassium on Determination of Manganese and Iron

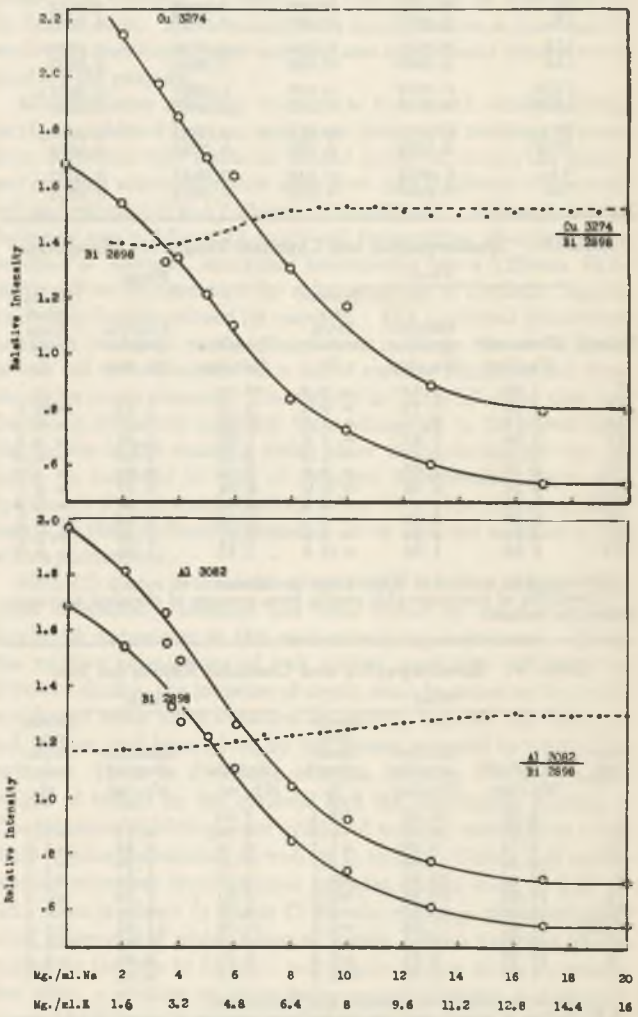


Figure 3. Effect of Sodium and Potassium on Determination of Copper and Aluminum

Within this range sodium and potassium may vary considerably without changing the spectral ratio of the elements studied.

The efficiency of the buffer solution was tested at two dilutions of feces samples (Table III). In the A samples, 125 mg. of the ash were dissolved in 25 ml. of buffer. In the B samples 250 mg. of the same ashed feces were dissolved in 25 ml. of solution. The extraneous elements calcium, magnesium, and phosphorus are present in B solutions in double the amounts in the A samples. That doubling the amounts of these extraneous elements has little effect on the final result is shown by the fact that determinations on the B solutions give results for manganese, iron, aluminum, and copper twice as large as those obtained with the A samples (assuming ±5% as the error of the analysis).

Standard solutions were made, keeping the sodium and potassium at 12 and 9.6 mg. per ml., respectively. From these, working curves were derived. The internal standard, bismuth, was held constant at 0.45 mg. per ml. To reduce the cyanogen bands ammonium chloride was added, as advocated by Ewing, Wilson, and Hibbard (*6*). The trace elements, manganese, iron, aluminum, copper, tin, and lead were made to vary over sufficient range to produce a wide range of densities on the photographic plate. All other constituents were held constant and were present in amounts corresponding to those in the biological samples.

Table III. Results of Fecal Analysis at Two Dilutions

Sample	Manganese	Iron	Aluminum	Copper
	Milligrams per milliliter			
IA	0.0028	0.016	0.0040	0.0031
IB	0.0063	0.031	0.0075	0.0062
IIA	0.0025	0.014	0.0046	0.0028
IIB	0.0053	0.030	0.0080	0.0053
IIIA	0.0032	0.016	0.0037	0.0031
IIIB	0.0078	0.032	0.0070	0.0057
IVA	0.0033	0.015	0.0036	0.0026
IVB	0.0076	0.032	0.0073	0.0056
VA	0.0032	0.016	0.0041	0.0027
VB	0.0057	0.031	0.0072	0.0052

Table IV. Spectrographic and Chemical Results for Manganese

	Food			Feces		
Sample	Chemical[a]	Spectrographic	Deviation from mean[b]	Chemical[a]	Spectrographic	Deviation from mean[b]
	Mg./day	*Mg./day*	%	*Mg./day*	*Mg./day*	%
I	1.93	1.91	± 0.5	2.07	....	.....
II	1.83	1.71	± 3.4	2.12	1.71	±10.7
III	2.12	2.11	± 0.2	2.23	2.21	± 0.4
IV	1.94	1.87	± 1.8	1.95	2.04	± 2.2
V	2.16	2.11	± 1.2	2.25	1.97	± 6.6
VI	2.06	2.00	± 1.5	2.12	2.04	± 1.9
VII	2.29	2.03	± 6.0	2.04	2.17	± 3.1
VIII	2.18	2.06	± 2.8	2.44	2.39	± 1.0
IX	2.25	2.02	± 5.4	2.16	2.15	± 0.2
X	1.94	1.64	± 8.4	2.21	2.18	± 0.7
XI	2.04	1.65	±10.6	1.91	1.99	± 2.0

[a] Obtained by method of Willard and Greathouse (*19*).
[b] Deviation of spectrographic results from average of chemical and spectrographic values.

Table V. Spectrographic and Chemical Results for Iron

	Food			Feces		
Sample	Chemical[a]	Spectrographic	Deviation from mean[b]	Chemical[a]	Spectrographic	Deviation from mean[b]
	Mg./day	*Mg./day*	%	*Mg./ml.*	*Mg./ml.*	%
I	8.85	10.47	±8.4	7.34	....	....
II	8.93	9.53	±3.2	9.86	8.11	±9.7
III	9.70	9.46	±1.2	9.18	8.52	±3.7
IV	12.80	11.74	±4.3	7.42	7.59	±1.1
V	13.46	13.49	±0.1	8.19	8.44	±1.5
VI	10.89	10.94	±0.2	7.31	7.30	±0.1
VII	10.18	11.62	±6.6	8.18	9.31	±6.5
VIII	11.82	11.93	±0.5	10.05	11.81	±8.0
IX	10.22	10.50	±1.4	7.52	8.84	±8.1
X	10.17	9.71	±2.3	8.20	8.80	±3.5
XI	7.96	8.48	±3.2	7.61	9.04	±8.6

[a] Obtained by method of Hummel and Willard (*9*).
[b] Deviation of spectrographic results from average of chemical and spectrographic values.

Table VI. Aluminum, Copper, Tin, and Lead in Food and Feces

	Food				Feces			
Sample	Aluminum	Copper	Tin	Lead	Aluminum	Copper	Tin	Lead
	Milligrams per day							
I	4.00	2.19	1.33	0.58	....	....	....	....
II	3.39	1.77	1.52	0.56	1.92	1.68	1.18	0.36
III	2.45	3.79	1.79	0.52	2.94	1.86	1.41	0.28
IV	2.98	3.20	3.32	0.52	2.35	1.50	1.74	0.26
V	2.03	3.07	1.54	0.62	1.71	1.81	1.73	0.34
VI	1.58	3.05	0.92	0.45	1.59	1.50	1.00	0.25
VII	3.11	5.66	0.77	0.67	2.48	1.87	1.04	0.26
VIII	2.31	3.69	0.72	0.62	2.47	2.11	0.75	0.40
IX	2.62	4.15	0.70	0.52	2.10	1.96	0.58	0.28
X	2.84	1.93	0.75	0.66	2.30	1.71	0.66	0.69
XI	1.74	2.31	0.76	0.52	2.54	1.76	0.65	0.30

The buffer solutions permitted the same working curves to be used for both food and feces samples.

RESULTS

Manganese and iron in the food and feces samples were determined by both chemical and spectrographic methods. The chemical method of Willard and Greathouse (*19*) was used for the determination of manganese, with slight modifications. Iron was determined by the *o*-phenanthroline method of Hummel and Willard (*9*). Tables IV and V compare the data obtained by the two methods. Table VI gives the results for aluminum and copper in food and feces samples (obtained simultaneously with manganese and iron). Spectrographic results for tin and lead, determined on the same ash solution but under the optical conditions described in the procedure, are also given.

DISCUSSION

The buffer solution described eliminates the effect of variable amounts of sodium, potassium, calcium, and magnesium, and, in addition, almost entirely removes the high background which is characteristic of a direct current carbon arc method. Only a small background correction was necessary with the copper line 3274 Å. All other lines used were completely free of background. This feature of the buffer is applicable in other spectrographic methods where maximum sensitivity is not demanded. The buffer suppresses the lines of a number of elements; however, the elements studied were present in sufficient amounts to bring their line densities within the optimum range, with the exception of tin and lead. The buffer solutions when used in the manner described permit the same set of working curves to be used for both food and fecal samples. This fact eliminates an important source of error which would arise when separate working curves are necessary for each of the two types of samples, for in determining the amount of a particular metal absorbed by man the absorption value is obtained by difference. Thus, the combined errors in two separately determined sets of working curves are eliminated by the buffer solution which permits the analysis of both types of samples from one standardization.

The reproducibility of the spectrographic results for a single determination is about ±10% of the amount present. However, each ash solution was run in triplicate and the average of the three determinations treated as a single result. The same process was repeated until a check between results was obtained. Thus, for the values reported, at least six determinations were made with each ash solution. Treated in this manner the reproducibility is about ±5% of the amount. Work is continuing on the same samples in an effort to study the metabolism of still other trace elements and to extend the application of the spectrograph in this type of analysis.

LITERATURE CITED

(1) Brode, W. R., "Spectrochemical Analysis", 2nd ed., New York, John Wiley & Sons, 1943.
(2) Cholak, J., IND. ENG. CHEM., ANAL. ED., 7, 287 (1935).
(3) Cholak, J., Hubbard, D. M., and Story, R. V., *Ibid.*, 15, 57 (1943).

(4) Cholak, J., and Story, R. V., *Ibid.*, 10, 619 (1938).
(5) Duffendack, O. S., Wiley, F. H., and Owens, J. S., *Ibid.*, 7, 410 (1935).
(6) Ewing, D. T., Wilson, M. F., and Hibbard, R. P., *Ibid.*, 9, 410 (1937).
(7) Fox, H. M., and Ramage, H., *Nature*, 126, 682 (1930).
(8) Harrison, G. R., "Massachusetts Institute of Technology Wavelength Tables", New York, John Wiley & Sons, 1939.
(9) Hummel, F. C., and Willard, H. H., IND. ENG. CHEM., ANAL. ED., 10, 13 (1938).
(10) Hurter, F., and Driffield, V. C., *J. Soc. Chem. Ind.*, 9, 455 (1890).
(11) Lamb, F. W., IND. ENG. CHEM., ANAL. ED., 13, 185 (1941).
(12) Lundegårdh, H., *Kgl. Landtbruks-Akad. Handl. Tid.*, 75, 241 (1936).
(13) Lundegårdh, H., and Bergstrand, H., *Scientiarium Upsaliensis*, 12, 1 (1940).
(14) Macy, I. G., "Nutrition and Chemical Growth in Childhood, Vol. I, Evaluation", Springfield, Ill., Charles C Thomas, 1942.
(15) *Ibid.*, Vol. II, "Original Data", 1946.
(16) Pierce, W. C., and Nachtrieb, N. H., IND. ENG. CHEM., ANAL. ED., 13, 774 (1941).
(17) Smith, S. J., *Biochem. J.*, 25, 2162 (1931).
(18) Staud, A. H., and Ruehle, A. E., IND. ENG. CHEM., ANAL. ED., 10, 59 (1938).
(19) Willard, H. H., and Greathouse, L. H., *J. Am. Chem. Soc.*, 39, 2366 (1917).

A PRELIMINARY report on this work was given before the Division of Analytical and Micro Chemistry at the 107th Meeting of the AMERICAN CHEMICAL SOCIETY, Cleveland, Ohio. A portion of a Ph.D. thesis presented to the Graduate Faculty of Michigan State College by James K. Brody.

Quantitative Determination of Caramel

In Wine, Distilled Spirits, Vinegar, and Vanilla Extract

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A combined qualitative and quantitative method is presented by which caramel can be precipitated and separated from other coloring matter in wine, distilled spirits, vinegar, and vanilla extract. The identity of the caramel can be corroborated by known methods and the intensity of color determined by a tintometer. By use of tintometer readings, the caramel can be calculated as per cent of color or per cent by weight or volume in a liquid.

CARAMEL is extensively used as a coloring agent in beverages and food products. Manufactured by heating sugar or hydrolyzed starch until the sweet taste is destroyed and a uniform, dark-brown, viscous mass is formed, caramel is said to be a carbohydrate, having the formula $(C_{12}H_{18}O_9)_x$ (*6*). Von Elbe (*8*) after investigating sucrose caramel stated that "caramel consists of a mixture of colorless compounds and a dark-brown, 'humic' substance which shows the properties of a lyophobic colloid". That statement has been corroborated in this laboratory and work on caramel derived from other sugars shows that most varieties which have been thoroughly dried are insoluble in water, acid solutions, and alkaline solutions.

The principal varieties in commerce are acid proof, nonacid proof, foaming, malt, and baker's and confectioner's caramels. Another variety is sometimes found in concentrated grape must, or grape concentrate which has been heated at such a high temperature during evaporation of the juice that some of the sugar has caramelized. Large quantities of grape concentrate are used in the manufacture of wine, and since Internal Revenue laws prohibit the presence of any form of caramel in standard wine, it is necessary to have a method for detecting all varieties of caramel.

The literature contains many methods for detecting caramel in beverages and food, but no quantitative method. The experience in this laboratory, where a large part of the work involves wine, is that none of the published tests is satisfactory for all kinds of wine and distilled spirits, particularly grape concentrate and the wine made from it. The fact that caramel may be made partly insoluble in acid and alkaline solutions suggested the possibility of using such a reaction as the basis for a method for obtaining caramel free from other coloring matter. Common reagents such as ethers, alcohols, etc., which are used in some of the known methods, were tried without success; the caramel was not completely precipitated or it contained other coloring matter. Some of the chemicals, such as tartrates and sulfites, which are present in wine or are used in its manufacture were tried and it was found that the combinations used in the present method precipitated all the caramel without including other coloring matter.

The method was developed primarily for the analysis of wine, but is applicable to distilled spirits, vinegar, and vanilla extract. Since the caramel is removed completely and in a pure state from the liquid by the method of analysis, and from a caramel-free liquid no color is removed, a brown precipitate is proof of the presence of caramel. If desired, its identity can be corroborated by known tests. For a quantitative determination a colorimetric method is considered most suitable and a Lovibond tintometer is used for the purpose.

All tintometer readings were made at a north window, using daylight reflected from an opal glass plate. All readings of more than 20 brown were made on diluted solutions, except the standard caramel solutions which were read in a 0.156-cm. ($^1/_{16}$-inch) cell and calculated to a 1.25-cm. (0.5-inch) cell. Although manufacturers use a 2.5-cm. (1-inch) cell for readings of color in the analysis of caramel, analytical laboratories use a 1.25-cm. (0.5-inch) cell as the standard for color readings of alcoholic liquors and other liquids colored by caramel. The Lovibond tintometer has been criticized on the ground that readings cannot be made in one cell and calculated to a cell of another thickness and that the slides are inaccurate. The figures in Table III show that the Lovibond slides are accurate for readings up to 20 brown and the figures in the example below show that calculations can be made on readings in cells of different thicknesses. Beyer (*4*) has shown that caramel solutions follow Beer's law and that readings with the Lovibond tintometer are as accurate as those made with a photometer.

Allen (*1*) states in connection with color work on indigo, "The most satisfactory solution has been found by employing the Lovibond tintometer as the color-measuring instrument. Since the relative proportions of red, yellow, and blue will vary in different shades, the measure of depth must be taken as the total number of color units obtained by adding together the units of red, yellow, and blue, given by the glasses required to match the pattern. There is a definite relation between the percentage weight of indigo on the material and the tintometric reading." The principle of adding color units and making calculations with them applies to caramel as well as to indigo. Unless it is colorless, all wine free from caramel contains brown color as well as red. This is shown in Table IV by the readings of the original wine, every one of which contains brown. Even varieties which appear to the eye to be pure red require brown slides to match the color, a striking example being sample 140,309, a dark red claret which contains almost as much brown as red. If caramel is added to wine, the caramel recovered by the method of analy-

sis, and its color determined, the sum of the brown and red slides of the Lovibond scale divided by the sum of the brown and red slides for the reading of the original sample containing caramel multiplied by 100 equals the per cent of caramel color in the total color, because the readings are in units of color and they can be compared as any other units. To illustrate the method for calculating the per cent of caramel in a liquid, the following example is given, using a dark red Burgundy wine to which caramel was added and recovered by the method of analysis, the color readings being made in a Lovibond tintometer.

A. Wine

$\frac{4.0 \text{ brown}}{5.4 \text{ red}}$ in 1/16-inch cell $\Rightarrow$ $\frac{32.0 \text{ brown}}{43.2 \text{ red}}$ in 0.5-inch cell

B. Wine and water

22 ml. of wine and 3 ml. of water.

$\frac{3.5 \text{ brown}}{4.4 \text{ red}}$ in 1/16-inch cell $\Rightarrow$ $\frac{28.0 \text{ brown}}{35.2 \text{ red}}$ in 0.5-inch cell

C. Caramel and water

3 ml. of standard caramel solution and 22 ml. of water.

$\frac{12.0 \text{ brown}}{0.8 \text{ red}}$ in 0.5-inch cell

D. Wine and Caramel

22 ml. of wine and 3 ml. of standard caramel solution

$\frac{5.0 \text{ brown}}{4.6 \text{ red}}$ in 1/16-inch cell $\Rightarrow$ $\frac{40.0 \text{ brown}}{36.8 \text{ red}}$ in 0.5-inch cell

E. 3 ml. of caramel added to 22 ml. of wine and recovered by the method of analysis. Final solution diluted to 25 ml. for reading.

$\frac{10.5 \text{ brown}}{0.6 \text{ red}}$ in 0.5-inch cell

Reading of B + reading of C = reading of D, wine and caramel before analysis.

$\frac{28.0 \text{ brown}}{35.2 \text{ red}} + \frac{12.0 \text{ brown}}{0.8 \text{ red}} = \frac{40.0 \text{ brown}}{36.0 \text{ red}}$

Reading of B + reading of E = reading of wine and recovered caramel

$\frac{28.0 \text{ brown}}{35.2 \text{ red}} + \frac{10.5 \text{ brown}}{0.6 \text{ red}} = \frac{38.5 \text{ brown}}{35.8 \text{ red}}$

F. There is a reduction of 12.5% of color in the method; therefore the reading is corrected by dividing by 87.5 and multiplying by 100.

$\frac{10.5 \text{ brown}}{0.6 \text{ red}} \div 87.5 \times 100 = \frac{12.0 \text{ brown}}{0.7 \text{ red}}$

Reading of B + reading of F = reading of wine + reading of caramel recovered by analysis.

$\frac{28.0 \text{ brown}}{35.3 \text{ red}} + \frac{12.0 \text{ brown}}{0.7} = \frac{40.0 \text{ brown}}{35.9 \text{ red}}$ = D after analysis

The reading $\frac{12.0 \text{ brown}}{0.7 \text{ red}}$ or total of 12.7 units + $\frac{40.0 \text{ brown}}{36.8 \text{ red}}$ or total of 76.8 units × 100 = 16.5%. The caramel color is 16.5% of the total color in the wine containing caramel.

As an example of the method for calculating the approximate per cent by weight or volume of caramel in a liquid, suppose that a sample from a 50-gallon barrel of brandy is analyzed by the method and the color reading of the recovered caramel is $\frac{10.6 \text{ brown}}{0.4 \text{ red}}$ a total of 11.0 units in a 0.5-inch cell of the Lovibond tintometer.

Correcting for the 12.5% reduction in color due to the method

$$11.0 \div 87.5 \times 100 = 12.6 \text{ units}$$

Assume that the caramel in the brandy had the composition of the average shown in Table I. Since 0.10 gram per 100 ml. of the average caramel reads $\frac{9.6 \text{ brown}}{0.6 \text{ red}}$ a total of 10.2 units, the quantity required to read 12.6 units is

$$10.2:12.6 = 0.10:X \qquad X = 0.123 \text{ gram}$$

The total solids of the average caramel, 67.5%, is equivalent to a specific gravity of 1.33. The volume of caramel per 100 ml. which has a reading of 12.6 total units is

0.123 ÷ 1.33 = 0.092 ml.
0.092 ml. per 100 ml. is per cent by volume
0.092% of 1 gallon or 128 fluid ounces = 0.118 fluid ounces
0.118 × 50 = 5.9 fluid ounces of caramel in the 50-gallon barrel of brandy

Table I. Analysis of Caramel Samples as Received from Manufacturers

Sample No.	Mfgr.	Variety	Moisture % by Weight	Solids, % by Weight	Color Reading of 0.10 Gram of Caramel in 100 Ml. of Water, 0.5-Inch Cell
140,409	A	No claim	25.9	74.1	11.0 brown 0.8 red
141,730	A	No claim	37.7	62.3	11.0 brown 0.8 red
140,414	B	Acid-resistant	31.1	68.9	9.5 brown 0.8 red
141,729	B	No claim	38.1	61.9	9.0 brown 0.8 red
140,458	C	Malt	29.3	70.7	9.0 brown 0.8 red
140,461	D	Acidproof	22.1	77.9	11.0 brown 0.8 red
141,770	D	Foaming	31.3	68.7	11.0 brown 0.4 red
141,747	E	Bakers and confectioners	29.4	70.6	9.0 brown 0.8 red
140,471	E	No claim	34.2	65.8	8.5 brown 0.8 red
141,766	F	Extra strong	34.1	65.9	11.0 brown 0.4 red
141,767	F	Foaming	41.8	58.2	11.5 brown 0.4 red
141,832	G	Not acidproof	37.2	62.8	8.0 brown 0.4 red
140,413	G	Acid-resistant	27.7	72.3	8.0 brown 0.4 red
140,396	H	No claim	28.4	71.6	8.5 brown 0.4 red
140,397	I	No claim	28.1	71.9	7.5 brown 0.4 red
140,463	J	No claim	43.7	56.3	9.0 brown 0.8 red
		Av.	32.5	67.5	9.6 brown 0.6 red

Internal Revenue regulations permit a maximum of 6 fluid ounces of caramel to be added to 50 gallons of brandy. Therefore, in the brandy of unknown origin mentioned above the quantity of caramel is within the limits of the regulations.

In addition to the chemical reagents used in the method, there is another substance necessary for the precipitation of caramel, the nature of which is at present unknown. It is found in all wine, vinegar, and vanilla extract so far analyzed, but is not present in distilled spirits or caramel solutions and it must be furnished from some other source, for without it, caramel cannot be recovered quantitatively by this method from distilled spirits and caramel solutions. If a caramel in a 10 to 21% alcohol solution only is analyzed by the method for wine, not more than 50% of the caramel is recovered because of lack of the ingredient mentioned.

In the search for a substance which might contain this necessary precipitating material, several varieties of sugar were tried. When dextrose was used, the recovery of caramel was still only 50%, with honey 65%, with sucrose 75%, with sorbitol or mannite 85%, with maple sugar 90%, and with light brown granulated cane sugar 100%. All grades of brown sugar do not contain caramel. The one used in this work was obtained in one-pound packages at a grocery store; it was found to be caramel-free and very light in color, comparable to Mulliken's Color Chart A, yellow tint 2 (7). It should be tested by the method of analysis to determine its freedom from caramel, by the use of 3 grams in enough 10 to 21% alcohol to make a volume of 25 ml. During the search for this ingredient, gelatin and its hydrolysis products were studied, but it was found that they precipitated not only all of the caramel but also wine and fruit colors. Although sometimes used as a clarification medium in wine manufacture, gelatin has never been noticed in wine because of its insolubility in alcoholic liquids. Pectin, when added to wine, formed during the analysis a mass of stringy material or a curdy

precipitate depending on the quantity added, which carried wine color with it and ruined the determination. However, pectin has never been found in grape wine and only in small quantities in a few samples of abnormal fruit wine and in a concentrate made from black grapes. If present in wine, it can be removed from the caramel precipitate by the acetone–hydrochloric acid process described below. Dextrin in any appreciable quantity was found to collect on the sides of the precipitating cylinder as a white sticky mass, but it was encountered only while endeavoring to adapt the method to the analysis of beer. The method can be used for the determination of caramel in beer but it is most unsatisfactory and is not recommended.

STANDARD CARAMEL SOLUTIONS

For use in developing the method, 16 samples of caramel obtained from 10 manufacturers were analyzed for moisture, solids, and tinctorial strength in order to compare their quality. The tinctorial strength was determined in a solution of 0.10 gram of caramel in 100 ml. of water, using a 0.5-inch cell in a Lovibond tintometer, matching the color with brown slides series 52 and red slides series 200. The results are shown in Table I.

Color is the only basis on which the various brands of commercial caramel can be compared because they vary in moisture, solids, ash, etc.; so if all the samples are adjusted to the same depth of color they are comparable in quality. Since it was found that diluted caramel solutions were decomposed by yeasts and molds, even when kept in a refrigerator, freshly prepared standard solutions were used in all determinations.

Each sample of caramel was diluted with sufficient water to yield a solution of tinctorial strength corresponding to a reading of $\frac{12.0 \text{ brown}}{0.4 \text{ red}}$ to $\frac{12.5 \text{ brown}}{0.8 \text{ red}}$ in a $^1/_{16}$-inch cell. This was adopted as the standard and it made all the samples comparable. The standard solutions were analyzed for solids and ash (Table II). These figures may be of value in detecting adulterated caramel. If a sample is diluted sufficiently to have a color reading of 12.0 to 12.5 brown in a $^1/_{16}$-inch cell and its solid content is materially less than the minimum shown in Table II, the inference is very strong that the sample contains color other than caramel.

Table II. Analysis of "Standard Solutions of Caramel"

(Color readings of 12.0 to 12.5 brown in $^1/_{16}$-inch cell)

Sample No.	Mfgr.	Solids *G./100 ml.*	Ash *G./100 ml.*
140,409	A	0.6725	0.0186
141,730	A	0.6200	0.0130
140,414	B	0.6585	0.0122
141,729	B	0.6530	0.0231
140,458	C	0.8135	0.0056
140,461	D	0.7155	0.0190
141,770	D	0.6172	0.0250
141,747	E	0.7580	0.0200
140,471	E	0.7332	0.0025
141,766	F	0.6500	0.0672
141,767	F	0.5155	0.0358
141,832	G	0.7110	0.0070
140,413	G	0.9130	0.0100
140,396	H	0.9042	0.0962
140,397	I	0.9130	0.0625
140,463	J	0.6230	0.0520
	Av.	0.7169	0.0293

Various volumes of the standard solutions having color readings of $\frac{12.0 \text{ brown}}{0.4 \text{ red}}$ to $\frac{12.5 \text{ brown}}{0.8 \text{ red}}$ in a $^1/_{16}$-inch cell were diluted to 25 ml. and tintometer readings made in a 0.5-inch cell; 1.5 ml. of such a solution diluted to 25 ml. read $\frac{6.0 \text{ brown}}{0.4 \text{ red}}$ in a 0.5-inch cell. However, when 1.5 ml. of a standard solution were diluted to 25 ml. and treated as in the last part of the method of analysis, beginning with the sentence "Transfer the paper and precipitate to a 150-ml. beaker." and finally read in a 0.5-inch cell, the color was invariably $\frac{5.0 \text{ brown}}{0.2 \text{ red}}$ to $\frac{5.5 \text{ brown}}{0.4 \text{ red}}$ instead of $\frac{6.0 \text{ brown}}{0.4 \text{ red}}$

This procedure shows that there was a reduction in color, due not to a loss of caramel precipitation in the main part of the method but to the treatment of the precipitated caramel after it was removed from the rest of the sample. The reduction in color, averaging 12.5%, is characteristic of the method, and is consistent as shown by the readings in Table III and the other tables.

Table III. Color Reading of Standard Caramel Solutions Diluted with Water to 25 Ml.[a]

Volume of Solution, Ml.	Color Reading: Untreated solution, 0.5-inch cell	Color Reading: Treated solution, 0.5-inch cell
0.75	3.0 brown 0.2 red	2.5 to 2.75 brown 0.2 to 0.2 red
1.5	6.0 brown 0.4 red	5.0 to 5.5 brown 0.2 to 0.4 red
3.0	12.0 brown 0.8 red	10.0 to 11.0 brown 0.6 to 0.8 red
6.0	23.0 to 24.0 brown 1.0 to 1.4 red	20.0 to 22.0 brown 1.0 to 1.4 red

[a] Standard caramel solutions were treated by the last part of the method of analysis beginning with "Transfer the paper and precipitate to a 150-ml. beaker". An average of 12.5% reduction in the color reading was caused by the treatment.

DETERMINATION OF CARAMEL

METHOD FOR WINE. *Reagents.* Powdered boric acid, U.S.P. Powdered citric acid, U.S.P.

Powdered potassium bitartrate, C.P. Powdered tartaric acid, C.P.

Powdered sodium bisulfite, reagent grade, minimum 95% $NaHSO_3$ (not sodium metabisulfite)

Sodium hydroxide, C.P. Ether, C.P. Acetone, C.P. Alcohol, U.S.P.

Boric-Citric Acid Solution. To 100 ml. of 95% alcohol, add 6 grams of boric acid and 2 grams of citric acid, warm to 55° C. for solution, and cool to room temperature.

Precipitating Solution. To 56 ml. of the alcoholic boric-citric acid solution in a 100-ml. cylinder, add 19 ml. of ether and 25 ml. of acetone and mix. Prepare just before use.

Alcoholic Sodium Hydroxide Solution. To 100 ml. of alcohol which is practically aldehyde-free add 4 grams of sodium hydroxide dissolved in 4 ml. of water, mix, filter, and keep in a tightly stoppered bottle.

Apparatus. Glass-stoppered cylinder graduated to 100 ml. and capable of holding 130 ml.

Procedure. Place 25 ml. of wine, containing 10 to 21% of alcohol by volume and not more than 20% of solids, in the specified glass-stoppered cylinder. Add in the order named, shaking after each addition, 0.3 gram of potassium bitartrate, 0.1 gram of tartaric acid, and 0.4 gram of sodium bisulfite. Allow the mixture to stand for 10 minutes and add 100 ml. of the precipitating solution. Shake the cylinder vigorously for 1 or 2 minutes, removing the stopper every 15 or 20 seconds to release the pressure and stand overnight for complete precipitation of the caramel. Place in a Gooch crucible a mat of paper pulp $^1/_{16}$ inch or less in thickness, rinse it with alcohol using suction, then decant through it the liquid in the cylinder until approximately 10 ml. remain. Mix the liquid and precipitate in the cylinder and pour rapidly through the Gooch. Rinse the cylinder several times with 5 to 10 ml. of alcoholic boric-citric acid solution, pouring the rinsings through the Gooch. Wash the precipitate in the Gooch with 25 ml. of hot alcoholic boric-citric acid solution (heated to boiling in a 600-ml. beaker on an electric hot plate to prevent ignition and swirling the beaker constantly to prevent bumping), followed by 10 ml. of 95% alcohol, then 10 ml. of 4% alcoholic sodium hydroxide solution, and finally by 10 ml. of 95% alcohol.

Transfer the paper and precipitate to a 150-ml. beaker, and wash the Gooch with 5 ml. of 0.5 *N* aqueous sodium hydroxide solution, followed by 15 ml. of water, adding the washings to the beaker. Test with litmus paper to be sure the solution is alkaline. Boil vigorously for several minutes to dissolve the caramel, stirring constantly to prevent bumping, and cool gradually to room temperature. Filter the contents of the beaker through a 9-cm. filter paper which has been wet with water, collecting the filtrate in a 50-ml. cylinder. Wash with water until the filtrate is colorless and measures approximately 23 ml., make faintly acid with *N* hydrochloric acid, and complete the volume to 25 ml. with water. Read the color of the caramel solution in a 0.5-inch cell in the Lovibond tintometer, correct it for the 12.5% manipulation loss, divide it by the color reading of the original wine, and multiply by 100 for the per cent of caramel color in the total color.

The analysis of grape concentrate for caramel coloring is more difficult than that of wine itself because the concentrate contains all the solids and vegetable color originally present in the grape

juice, much of which is removed in the process of manufacturing wine. The concentrate must be diluted with 10 to 21% alcohol and filtered before analysis. Difficulty may be experienced in filtering the concentrate even after diluting 2 to 5 ml. of the sample to 25 ml. For this reason 2 ml. of the concentrate are generally preferred. The use of suction or a centrifuge may not aid the filtration. The best procedure is to dilute 4 ml. of the concentrate to 50 ml. with 10 to 21% alcohol, add 1 gram of purified talc U.S.P., mix, and filter through a dry double filter paper. It may take several hours for this filtration, but the liquid must be clear; otherwise the method is a failure. Purified talc U.S.P. is inert and no caramel will be adsorbed by it when the quantity used is small.

Some varieties of very dark colored grapes produce concentrate which is very different in composition from the ordinary varieties. It is almost black in color and, because of being unfermented, contains an excess of iron which, during the analysis, precipitates an iron color complex noticeable as a red contamination in the caramel. The coloring matter is not removed by the regular method of analysis and a special procedure is necessary to eliminate it. No wine or ordinary grape concentrate has been analyzed which caused trouble and this extra step does not need to be taken unless the caramel precipitate is abnormal because of its color or other unusual appearance which will be apparent to an experienced analyst. The procedure is as follows:

Prepare an acetone–hydrochloric acid mixture containing 25 to 29% acid by volume by pouring 15 ml. of C.P. acetone in a 25-ml. cylinder and adding hydrochloric acid (1.18 sp. gr.) to the 20- or 21-ml. mark. Pour the mixture into a small beaker, cool it in ice water to 20° to 25° C., and use it immediately at that temperature. This volume, which is sufficient for one test, should be measured accurately because experiments have proved that caramel is soluble in stronger acid. However, the mixture of the strength specified will break up the iron color complex without dissolving the caramel as precipitated by the method. At the point in the method of analysis, beginning with the sentence "Transfer the paper and precipitate to a 150-ml. beaker.", fill the Gooch half full of the acetone–hydrochloric acid mixture, continue the suction until a drop of liquid is drawn through, discontinue the suction for a minute to allow the liquid to penetrate the precipitate, suction off the liquid, and wash thoroughly with alcohol. Repeat the process using alcoholic sodium hydroxide and wash with alcohol.

Replace the Gooch in the suction flask, with a second one containing a mat of paper pulp and with a small stirring rod transfer the mat and precipitate from the first Gooch to the second one. Fill the second Gooch half full of the acetone–hydrochloric acid mixture and, while holding the mat against the side of the crucible with a stirring rod, break it up with a second rod, without disturbing the lower mat. When it is disintegrated and saturated with the acetone–hydrochloric acid mixture apply suction, draw the liquid through, add the remainder of the mixture, suck it dry, wash with alcohol, discontinue the suction, add alcoholic sodium hydroxide, stir the loose pulp, suck it dry, and wash with alcohol. Place the mat and precipitate in a 150-ml. beaker, and wash both Gooch crucibles with 5 ml. of 0.5 *N* aqueous sodium hydroxide solution, followed by 15 ml. of water, adding the washings to the beaker. From this point follow the regular method. If it is necessary to reduce the volume of the caramel solution, evaporate it in an alkaline condition to less than 25 ml., make slightly acid, and complete the volume to 25 ml.

Table IV. Analysis of Grape, Berry, and Fruit Wine before and after Addition of Caramel

(0.5-inch cell)

Sample No.	Wine	Alcohol % by volume	Color Reading of Original Wine	Color Reading of Final Solution after Analysis of Original Wine	Volume of Standard Solution of Caramel Added to Original Wine Ml.	Color Reading of Final Solution Representing Recovered Caramel
139,476	Muscatel	20.0	7.5 brown 1.8 red	Colorless	1.5	5.5 brown 0.4 red
139,425	Muscatel	20.6	15.0 brown 10.0 red	Colorless	3.0	10.5 brown 0.8 red
137,827	Muscatel	18.2	5.75 brown 0.6 red	Colorless	0.75	2.6 brown 0.2 red
141,587	Muscatel	15.5	11.5 brown 0.6 red	Colorless	6.0	21.0 brown 1.2 red
141,696	Muscatel	19.5	22.5 brown 1.6 red	Trace	1.5	5.75 brown 0.6 red
134,428	Burgundy	12.0	24.0 brown 65.0 red	Colorless	1.5	5.5 brown 0.2 red
137,280	Burgundy	13.0	32.0 brown 96.0 red	Colorless	0.75	2.75 brown 0.2 red
140,878	Zinfandel	13.0	16.0 brown 103.0 red	Colorless	1.5	5.5 brown 0.2 red
140,309	Claret	13.5	16.0 brown 17.2 red	Colorless	3.0	10.0 brown 0.6 red
142,212	Chianti	13.1	12.0 brown 29.2 red	Colorless	0.75	2.75 brown 0.2 red
139,253	Port	23.6	16.0 brown 25.6 red	Colorless	3.0	10.0 brown 0.6 red
140,388	Tawny port	20.0	18.0 brown 4.0 red	Colorless	3.0	10.0 brown 0.6 red
139,182	Port	20.5	25.6 brown 29.6 red	Colorless	1.5	5.25 brown 0.4 red
140,337	Sherry	20.4	14.0 brown 1.4 red	Trace	1.5	5.25 brown 0.2 red
140,339	Sherry	20.0	18.0 brown 2.0 red	Colorless	3.0	10.0 brown 0.6 red
140,384	Sherry	20.5	10.0 brown 0.4 red	Colorless	0.75	2.6 brown 0.2 red
140,382	Sherry	20.25	16.0 brown 1.5 red	Trace	1.5	5.5 brown 0.5 red
142,736	Sherry	20.2	12.5 brown 0.8 red	Colorless	1.5	5.25 brown 0.4 red
139,475	Malvasia	21.4	16.0 brown 11.6 red	Colorless	6.0	20.5 brown 1.2 red
140,842	Angelica	20.8	15.0 brown 1.8 red	Colorless	3.0	10.5 brown 0.6 red
142,450	Tokay	20.2	7.5 brown 3.8 red	Colorless	0.75	2.75 brown 0.2 red
139,100	Alicante	20.6	18.0 brown 20.0 red	Colorless	1.5	5.5 brown 0.4 red
140,387	Chablis	12.8	5.5 brown 0.4 red	Colorless	0.75	2.6 brown 0.2 red
140,839	Sauterne	13.4	10.0 brown 0.8 red	Colorless	1.5	5.25 brown 0.4 red
141,920	Apple	20.0	7.0 brown 0.4 red	Colorless	3.0	10.25 brown 0.8 red
141,922	Loganberry	13.0	14.0 brown 43.2 red	Colorless	1.5	5.0 brown 0.4 red
141,921	Blackberry	13.0	26.0 brown 20.8 red	Colorless	0.75	2.6 brown 0.2 red
141,926	Currant	13.0	12.0 brown 9.6 red	Colorless	1.5	5.0 brown 0.6 red
148,169	Cherry	21.0	11.0 brown 2.2 red	Colorless	3.0	10.25 brown 0.8 red
140,555	Orange	19.0	3.0 brown 0.2 red	Colorless	1.5	5.0 brown 0.4 red
142,135	Peach	20.1	4.5 brown 0.2 red	Colorless	3.0	10.5 brown 0.6 red

METHOD FOR DISTILLED SPIRITS. *Reagents.* Ethyl acetate, C.P. Ammonium chloride, C.P. Commercial brown sugar (sucrose), granulated, caramel-free.

Reagents and apparatus listed under wine analysis.

Procedure. Place 25 ml. of the spirits in a separatory funnel, add 50 ml. of ether, shake vigorously for 1 minute to remove the alcohol, allow to settle for at least 15 minutes, draw off the lower aqueous layer into another separatory funnel, and discard the ether layer. Add to the aqueous solution 25 to 35 ml. of ethyl acetate, shake vigorously for 1 minute to remove uncharred oak tannin, allow to settle for at least 15 minutes, draw off the lower layer into the specified glass-stoppered cylinder, add 3 grams of brown sugar, fill to the 21-ml. mark with water, and complete the volume to 25 ml. with 95% alcohol. Mix the contents of the cylinder to dissolve the sugar and proceed as directed for wine. After the final solution has been acidified and made up to a volume of 25 ml., add 0.07 gram of ammonium chloride, mix, and allow to stand for at least 30 minutes. If any tannin from redwood or uncharred oak remains in the solution, it will be precipitated by the ammonium chloride. Filter through a 9-cm. filter paper, read the color of the filtrate in a 0.5-inch cell, and calculate the per cent of caramel in the original spirits as described under wine.

METHOD FOR VINEGAR. *Procedure.* Place 22 ml. of vinegar in the specified glass-stoppered cylinder and add 3 ml. of 95% alcohol. Proceed as directed for wine. Make the final volume up to 22 ml. for the color reading and calculate the per cent of caramel in the original vinegar as described under wine.

METHOD FOR VANILLA EXTRACT. *Procedure.* To 25 ml. of vanilla extract, add 25 ml. of water and 1 gram of purified talc and mix. Prepare a layer of paper pulp in a Gooch crucible using suction, and filter the vanilla mixture through it several times until a layer of talc is formed and the filtrate is clear. Place 25 ml. of the liquid in the specified glass-stoppered cylinder and proceed as directed for wine. Make the final volume up to 25 ml. and multiply the color reading by 2 or make it up to 12.5 ml. and use the direct reading. Calculate the per cent of caramel in the original vanilla extract as described under wine.

Table V. Analysis of Grape and Raisin Concentrates and Wine

(0.5-inch cell)

Sample No.	Grape and Raisin Concentrates	Color Reading: Concentrate before dilution	Color Reading: Final solution after analysis of diluted concentrate	Color Reading: Caramel calculated to undiluted concentrate
141,691	Raisin concentrate (5 ml. diluted to 25 ml. with 20% alcohol for analysis)	200.0 brown 12.0 red	3.5 brown 0.4 red	17.5 brown 2.0 red
137,872	Grape concentrate (same dilution)	375.0 brown 20.0 red	5.0 brown 0.4 red	25.0 brown 2.0 red
140,826	Red grape concentrate (same dilution)	20.0 brown 50.0 red	Colorless	....
140,827	Red grap concentrate (same dilution)	150.0 brown 200.0 red	Colorless	....
140,828	White grape concentrate (same dilution)	100.0 brown 6.0 red	Colorless	....
140,829	Red grape concentrate (same dilution)	40.0 brown 50.0 red	Colorless	....
	Overcooked Grape Concentrate[a]			
136,947	5 ml. concentrate diluted to 25 ml. with 20% alcohol	575.0 brown 40.0 red	30.0 brown 2.4 red	150.0 brown 12.0 red
136,947	2 ml. concentrate, diluted to 25 ml. with 20% alcohol	575.0 brown 40.0 red	11.5 brown 1.0 red	140.0 brown 12.5 red
	Wine Made from Concentrate 136,947			
	Wine	Alcohol by Volume, %	Color Reading before Analysis	Color Reading after Analysis
138,192[b]	Red port, no dilution	20.9	42.0 brown 9.6 red	5.0 brown 0.6 red
137,009[c]	Red port, no dilution	20.8	40.0 brown 8.8 red	3.5 brown 0.6 red

[a] Sample 136,947 was overcooked and contained a considerable quantity of caramel. The manufacturer blended it with wine in such small proportions he thought the color added by the caramel would be of negligible value and could not be detected. Two lots of blended wine, samples 138,192 and 137,009, were analyzed, the caramel was detected, and the quantity determined. The caramel color reading of the wine corrected for the reduction of 12.5% in the method, divided by the caramel color reading of the concentrate, corrected, multiplied by 100 equals the per cent of concentrate used in the wine.

[b] For sample 138,192, the calculation is

$(5.0 + 0.6) \div 87.5 \times 100 = 6.4$ corrected color reading for wine.

$(150.0 + 12.0) \div 87.5 \times 100 = 185.1$ corrected color reading for concentrate.

$6.4 \div 185.1 \times 100 = 3.5\%$ of concentrate in wine.

[c] For sample 137,009, a similar calculation shows presence of 2.5% of concentrate in wine.

DISCUSSION OF THE METHOD

The sample should be clear, filtered if necessary. If a sample of less than 25 ml. is used, it should be diluted with alcohol and water, so that the volume for analysis is 25 ml. containing 10 to 21% alcohol. The percentage of water is as important as that of any other reagent. Most inorganic salts, other than the reagents listed, such as common laboratory chemicals, are detrimental as they tend to disturb the chemical balance, preventing complete separation of the caramel and precipitating an excess of the reagents. Normal wine, distilled spirits, vinegar, and vanilla extract do not contain such salts in sufficient quantity to interfere with the method.

The ratio between the reagents used in the method is very definite and the quantities of all of them should be carefully measured, as a material change would interfere with the proper precipitation of the caramel. The quantities used are satisfactory for all types of dry and sweet wine, distilled spirits, vinegar, and vanilla extract. The difference in acidity between eastern and California wine was taken into consideration in fixing the quantity of tartaric acid to be used and the normal sulfite content of wine in fixing the quantity of sodium bisulfite. The potassium bitartrate, tartaric acid, and sodium bisulfite with the boric and citric acids not only aid in the precipitation of the caramel, but also form a mat on which the caramel settles, permitting its easy removal. The alcoholic boric-citric acid solution acts as a solvent for sugar and other solid matter; the more sugar there is in the sample the less cream of tartar and tartaric acid are precipitated, a point being reached at which neither reagent is precipitated and a sirupy mass is thrown down which makes the removal of the caramel from the cylinder exceedingly difficult. Therefore, the solids in the sample being analyzed should not exceed 20%.

All of the samples of caramel reduced Fehling's solution because of the presence of sugar, but in this method, all sugar is removed by the use of the alcoholic boric-citric acid solution and in no instance has the final caramel solution after analysis reduced Fehling's solution. Because of this fact, in the final step of the analysis the precipitated caramel can be boiled in aqueous solution with alkali to dissolve it without any possibility of increasing the caramel.

All caramel color readings should be made in a solution which is slightly acid because the color is darker in an alkaline than in an acid condition, this darkening being due to a large increase in red color with only a slight increase in the brown. If it is desired to evaporate a caramel solution after precipitation by this method, it should be done in an alkaline condition because non-acid proof caramel may be precipitated in a liquid which is more than slightly acid.

APPLICATIONS

Many varieties of wine, including grape, berry, and fruit, were analyzed by the method and when no caramel was present, the final solution was colorless, except in a few instances when there was a trace of color, which it was impossible to remove by any variation in the method. In other samples of the same varieties of wine, the final solutions were colorless. For these reasons it was concluded that the slight color was due to a small quantity

Table VI. Analysis of Distilled Spirits before and after Addition of Caramel

(0.5-inch cell)

Sample No.	Variety	Proof	Marsh Test (2)	Cyclohexanol Test (5)[b]	Color Reading of Original Spirits	Color Reading of Final Solution after Analysis of Spirits	Standard Solution of Caramel Added to Original Spirits, Ml.	Color Reading of Final Solution Representing Caramel Recovered
131,905	Whisky	100.0	Negative	Negative	15.0 brown 0.4 red	Colorless	1.5	5.0 brown 0.4 red
131,353	Brandy	100.0	Negative	Negative	10.0 brown 0.2 red	Colorless	3.0	9.75 brown 0.8 red
139,954	Scotch	86.8	Positive	Positive	8.5 brown 0.4 red	3.0 brown 0.2 red	3.0	13.0 brown 0.8 red
137,974	Rum, domestic	86.0	Negative	Negative	7.0 brown 0.4 red	Colorless	0.75	2.6 brown 0.2 red
101,989	Rum[a], imported	87.0	Positive	Positive	45.0 brown 4.0 red	16.0 brown 1.0 red	....	....
142,029	White oak chips	100.0	Positive	Negative	6.0 brown 0.2 red	Colorless	0.75	2.5 brown 0.2 red
142,028	Redwood chips	100.0	Trace	Trace	9.0 brown 1.4 red	Colorless	6.0	20.0 brown 0.8 red

[a] Labeled "Imported West Indies Rum, carefully distilled and aged 2.5 years in charred white oak casks". Rum was very dark in color and contained coal-tar dye, caramel, and oak wood color. Coal-tar dye extracted from rum in wool dyeing test was reddish-brown. Final solution containing caramel after analysis of rum was tested for coal-tar dye and result was negative, showing that dye in rum did not interfere with method for caramel.

[b] The cyclohexanol test is similar to Marsh test, but has the advantage that color from uncharred oak goes into the upper layer, whereas with the Marsh test the color from uncharred oak goes into the lower layer the same as caramel. The reagent is composed of 50 ml. of cyclohexanol, 50 ml. of methyl propyl ketone, 3 ml. of sirupy phosphoric acid (85%), and 3 ml. of distilled water.

of caramel incorporated in the wine by the use of caramelized concentrate as a blending agent. This conclusion was verified by combining and concentrating several of the solutions containing the color and applying the phenylhydrazine test (3) to them. In every instance a precipitate was obtained. Table IV shows the results of the analysis of the caramel-free wine, the volume of standard caramel solution added to the wine, and the tintometer reading of the recovered caramel. The smallest addition of standard caramel solution was 0.75 ml. and the largest 6.0 ml. in enough wine to make a volume of 25 ml. for analysis. The maximum of 6 ml. was taken because that dilution makes a liquid as dark as would ordinarily be used in any commercial wine. When a definite volume of a standard solution of caramel was added to each sample of wine, the color reading of the final solution containing the recovered caramel was the same as that of the standard caramel solution made up to 25 ml. after treatment as shown in the last column of Table III. When corrected for the average reduction in color of 12.5%, the recovery was 100% except in the case of the foaming type which was 80%. Foaming caramel is different from other varieties in composition and properties and the present work indicates that it may contain less actual caramel color and more brown color which is not caramel, thus accounting for the lower recovery value.

Some of the samples of sherry, such as No. 140,339, were extremely dark for sherry, but analysis disclosed no caramel, proving that deep color does not necessarily mean caramelization as a result of the baking of the sherry.

Several samples of grape concentrate and one of raisin concentrate were analyzed by the method. Two of them were found to contain caramel and the others had none (Table V).

Several samples of distilled spirits were analyzed by the method and in every instance in which no caramel was present, the final solution was colorless. When a definite volume of a standard solution of caramel was added to each sample, full recovery of the caramel w obtained. The Scotch whisk contained caramel and wh 1.5 ml. of a standard solution caramel were added to it, th final solution after analys contained all the added caram as well as that originally in th whisky.

Since most wine is stored i redwood containers and di tilled spirits in oak, it was d sired to know what effect th extractive matter of those con tainers would have on th analysis of liquor stored i them. Oak and redwood chip were soaked in 50% alcohol un til the liquids were dark be cause of the color extracted The two liquids were the analyzed by the method, i each case the final solution wa colorless, showing that neithe oak color nor redwood color in terferes with the separation o caramel. When caramel was added, full recovery was obtaine in each liquid.

Two samples of rum were analyzed, one of which was found t contain coal-tar dye, in addition to caramel. To investigate th effect of dyes on the method, experiments were performed, usin six kinds of coal-tar dyes and two of vegetable colors, all of whic made brown colored liquids in dilute solutions. Each of the eigh colors was added to a sample of wine or spirits which was the analyzed. In every instance, the final solution was colorless showing that the particular coal-tar and vegetable colors used did not interfere with the method for caramel. The results of the analysis of the distilled spirits are shown in Table VI.

Three samples of apple cider vinegar were analyzed by the method and in each one, the final solution contained a trace of color. Since the sweet cider and apple wine analyzed contained no caramel, it was concluded that the color in the vinegar was due to a small quantity of caramel which was formed in the cooking of the apple products before they were used in the vinegar process. This conclusion was verified by combining and concentrat-

Table VII. Analysis of Vinegar and Vanilla Extract before and after Addition of Caramel

(0.5-inch cell)

Sample No.	Material	Color Reading of Original Material	Color Reading of Final Solution after Analysis of Original Material	Standard Solution of Caramel Added to Original Material, Ml.	Color Reading of Final Solution Representing Recovered Caramel
140,362	Cider vinegar	10.0 brown 0.6 red	Trace	1.5	5.75 brown 0.4 red
141,945	Cider vinegar	13.5 brown 0.8 red	Trace	3.0	10.5 brown 0.6 red
142,278	Cider vinegar	14.0 brown 0.8 red	Trace	0.75	3.0 brown 0.2 red
142,191	Pure apple juice (plus 1 ml. of glacial acetic acid)	4.0 brown 0.6 red	Colorless	1.5	5.25 brown 0.4 red
131,658	Bourbon vanilla extract	136.0 brown 8.0 red	Colorless	1.5	5.25 brown 0.4 red
141,949	Tahiti vanilla extract	32.0 brown 1.6 red	Colorless	0.75	2.6 brown 0.2 red
141,948	Mexican vanilla extract	40.0 brown 1.6 red	Colorless	3.0	10.0 brown 0.6 red
141,947	Bourbon vanilla extract	48.0 brown 3.2 red	Colorless	6.0	21.0 brown 1.2 red

ing several of the solutions containing the color and applying the phenylhydrazine test (*3*) to them. In every instance a precipitate was obtained.

Four samples of genuine vanilla extract were analyzed by the method and in every instance the final solution was colorless, proving the absence of caramel. The results are shown in Table VII.

SUMMARY

Caramel in wine, distilled spirits, vinegar, and vanilla extract is precipitated by organic acids and solvents in a form easily freed from other coloring matter. The purified precipitate is caramel and its identity can be corroborated by the usual tests.

The quantity is determined in a Lovibond tintometer by color readings which are very accurate up to 20 units and when the manipulation loss in the method is corrected, the results of analysis are also very accurate.

The use of analytical results in making calculations is shown by the examples given for (1) per cent of caramel color in the total color, (2) per cent by weight or volume of caramel in a liquid, and (3) per cent of caramelized concentrate in a volume of wine (Table IV).

The method can be applied to all varieties of grape, fruit, and berry wine, distilled spirits, vinegar, vanilla extract, grape concentrate, and commercial caramel. It is not affected by substances usually present in those liquids, such as fruit and vegetable colors, wood extractive matter, or by certain coal-tar dyes.

LITERATURE CITED

(1) Allen's Commercial Organic Analysis, 5th ed., Vol. V, p. 384, Philadelphia, P. Blakiston's Sons & Co., 1927.
(2) Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis, 5th ed., p. 180 (1940).
(3) *Ibid.*, p. 252.
(4) Beyer, G. F., *J. Assoc. Official Agr. Chem.*, 26, 164 (1943).
(5) Clapp, H. L., Alcohol Tax Unit Laboratory, Philadelphia, Pa., unpublished.
(6) Hackh's Chemical Dictionary, 3rd ed., p. 165, Philadelphia, Blakiston Co., 1944.
(7) Mulliken, S. S., "Identification of Pure Organic Compounds", Vol. I, Color Standard Sheet A—Y—Tint 2, New York, John Wiley & Sons, 1904.
(8) Von Elbe, Guenther, *J. Am. Chem. Soc.*, 58, 600 (1936).

Determination of Itaconic Acid in Fermentation Liquors

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A method presented for the direct determination of itaconic acid in fermentation liquors containing other acids and glucose involves the use of aqueous bromine buffered at pH 1.2 to ensure the selective absorption of bromine by the itaconic acid.

DURING a recent study of itaconic acid production by strains of the mold *Aspergillus terreus*, a rapid and accurate method was developed for the determination of itaconic acid present in the fermentation liquors. This procedure involves a measurement of bromine absorption by itaconic acid in the presence of acid-buffered bromine water, and is based on the observation that, at pH 1.2, aqueous bromine reacts with itaconic acid equimolecularly but does not react with glucose, the principal interfering substance in fermentation media.

Heretofore, only slight consideration has been given to the determination of itaconic acid. Linstead and Mann (*4*), investigating the isomerization of unsaturated acids, used a bromometric method to determine itaconic acid in the presence of mesaconic acid. Calam, Oxford, and Raistrick (*1*) estimated the quantity of itaconic acid in fermentation liquors by simple alkaline titration and also by bromine absorption, using a method developed by Koppeschaar (*3*) for the determination of phenol. In the examination of fermentation liquors, the assumption that total acidity values represent itaconic acid may not be correct, because the presence of acids other than itaconic acid has been shown by Calam *et al.* (*1*), who noted that several strains of *A. terreus* produce succinic, fumaric, and oxalic acids. As used by the English group, Koppeschaar's method gave fairly good results because their fermentation substrates contained low concentrations of glucose (5% or less); however, this method will not give valid results in the presence of moderate quantities of glucose, owing to the reaction of this substance with bromine.

The method developed by the author is a refinement of Koppeschaar's procedure in that the bromine water is carefully acid-buffered, by means of a modification of Clark and Lubs' standard buffer mixtures (*2*), to ensure the selective absorption of bromine by itaconic acid in fermentation liquors containing glucose in concentrations as high as 15%.

REAGENT

Bromine	1.0 ml.
Potassium bromide	3.0 grams
Potassium chloride	1.87 grams
1.0 *N* HCl	48.5 ml.
Add water to	500 ml.

Dissolve the bromine and potassium bromide in a small amount of water before adding the other constituents. The resultant pH is 1.2 ± 0.1; no adjustment is required. Storage of the solution in a brown bottle in the refrigerator or in the dark slows the deterioration of bromine considerably.

METHOD

One to 2 ml. of fermentation liquor are pipetted into a 125-ml. iodine flask and to the sample are added 50 ml. of acid-buffered (pH 1.2) bromine water. The iodine flask stopper is water-sealed to prevent loss of bromine vapor. After 10 minutes' standing at room temperature, the flask is placed in an ice bath. After 5 minutes, 5 ml. of strong potassium iodide solution (50 grams of potassium iodide in 100 ml. of water) are poured into the well surrounding the stopper. The stopper is then lifted carefully, so that the vacuum created by the previous cooling sucks the potassium iodide solution into the flask.

After 10 minutes the released iodine is titrated with 0.1 *N* sodium thiosulfate, using starch indicator. The titer, *T*, of 50 ml. of bromine water (treated the same way as the sample) minus the titer, *B*, of unreacted bromine is equivalent to milliliters of 0.1 *N* itaconic acid; $(T - B)\ 0.1$ = milliequivalents of itaconic acid present or $(T - B)\ 0.0065$ = grams of itaconic acid in sample taken for analysis.

EXPERIMENTAL RESULTS

The reactivity of aqueous bromine with glucose and with itaconic acid is decreased upon lowering the pH. As shown in Table I, at pH 1.25 the bromine-glucose reaction cannot be detected during the first 15 minutes and is still negligible at 20 minutes. At pH 3.0 the reaction is faster and at pH 4.9 it is considerably accelerated.

At pH 1.25 the bromine–itaconic acid reaction is complete within 15 minutes. Results in Table II indicate the quantitative nature of this reaction.

A standard solution of 1 *N* itaconic acid was prepared containing 5% glucose. As shown in Table III, four determinations

Table I. Effect of pH and Time on Bromine-Glucose Reaction

(Sample 2 ml. of 5% glucose solution)

	Bromine Absorption, Grams of Itaconic Acid per 100 Ml.		
pH	5 min. at room temperature and 5 min. at 0° C.	10 min. at room temperature and 5 min. at 0° C.	15 min. at room temperature and 5 min. at 0° C.
1.25	0.00	0.00	0.13
3.00	0.00	0.07	0.26
4.90	2.80	3.31	3.96

according to the described method resulted in an average deviation of 1.5%.

The following substances in the concentrations indicated were found not to interfere, within the experimental accuracy of the method. They include most of the acids commonly found in mold fermentation liquors. Two-milliliter samples were analyzed in each case except that of glucose, in which case only 1 ml. was analyzed:

15% glucose
Saturated fumaric acid
1 *N* succinic acid
1 *N* lactic acid
1 *N* citric acid
1 *N* *d*-gluconolactone
1 *N* malic acid
1 *N* tartaric acid
1 *N* oxalic acid
1 *N* acetic acid
1 *N* aconitic acid

In Table IV are tabulated the results of analyses of several typical samples of fermentation liquor in which the total acid was determined by alkali titration and the itaconic acid by the described bromination procedure. The applicability of the bromination method to the analysis of liquors of widely varying composition is evidenced by sample analyses (Expt. 799-33) after 2 and 14 days' mold growth. Samples taken after 2 days' growth showed 11.1% residual glucose and very little total acid. Alkali titration, expressed as itaconic acid, was 0.091 gram per 100 ml., but no itaconic acid was indicated by bromine absorption. After 14 days' mold growth, the glucose concentration had fallen to 1.87% and the total acid had increased to 3.20 grams per 100 ml. (expressed as itaconic acid). The bromine value after 14 days indicated 2.97 grams of itaconic acid per 100 ml., representing purity index of 93.5. (Purity index $= \frac{\text{acid by bromine absorption}}{\text{acid by alkali titration}} \times 100$. This index is used as a measure of purity on the assumption that the unknown acids have approximately the same equivalent weight as itaconic acid.)

Sample 800-MC gave a bromine value of 3.06 grams of itaconic acid per 100 ml. as compared to a total acid value of 3.61 grams of acid per 100 ml. (purity index, 84.8). As a check on the titration method, 2.757 grams of crystalline itaconic acid were isolated from 100 ml. of sample 800-MC by concentration. This corresponds to a 90.1% recovery based on the bromine value. The remaining 10% of the itaconic acid in the mother liquor was recovered as the monohydrated calcium salt.

The discrepancy between total acidity and itaconic acid content (Table IV) as determined by the bromination method suggests the presence of appreciable quantities of acids other than itaconic in some of the culture liquors. Studies directed toward the isolation and identification of these acidic substances have been successfully prosecuted and will be reported in forthcoming communications from this laboratory.

Table II. Effect of Time on Bromine–Itaconic Acid Reaction at pH 1.25

(Sample: 1 ml. of 1 *N* itaconic acid, equivalent to 6.50 grams of acid per 100 ml.)

Reaction time at room temperature, minutes	5	10	15	25	2 hours	19 hours
Reaction time at 0° C., minutes	5	5	5	5	5	5
Bromine absorption, grams of itaconic acid per 100 ml.	6.21	6.50	6.57	6.54	6.60	6.65

DISCUSSION

The main advantage of the bromine absorption method is that itaconic acid can be determined directly in fermentation liquor containing glucose and many acids known to be mold metabolites. Aconitic acid, an unsaturated acid which has been suggested as a possible precursor of itaconic acid, does not interfere.

Table III. Determination of Itaconic Acid in Presence of Glucose

(Sample: 1 ml. of 1 *N* itaconic acid containing 5% glucose, equivalent to 6.50 grams of acid per 100 ml. Reaction time: 15 minutes, 10 min. at room temperature; 5 minutes at 0° C., pH 1.2)

Detn.	Bromine Absorption, Grams of Itaconic Acid per 100 Ml.
A	6.63
B	6.37
C	6.50
D	6.37

Another advantage is that bromine absorption values, when compared to total acidity values, indicate relative purities of experimental fermentations with respect to itaconic acid production. As has been shown, a low purity index indicates the presence of acids which do not react with bromine under the conditions of the method.

Table IV. Analyses of Fermentation Liquors

Expt. No.	Blank Titer		Blank Minus Titer	Itaconic Acid by Bromination	Total Acid by Alkali Titration	Purity Index	Residual Glucose
	Ml. of 0.1 N sodium thiosulfate		*Ml. of 0.1 N acid/2-ml. sample*	*G. of acid/100 ml.*	*G. of itaconic acid/100 ml.*		*G./100 ml.*
799-33							
2 days[a]	35.2	35.2	0	0	0.091	...	11.1
4 days	35.1	33.0	2.1	0.68	0.78	87.2	7.34
6 days	34.6	29.4	5.2	1.69	1.82	92.8	5.05
8 days	34.1	26.6	7.5	2.44	2.79	87.4	3.00
10 days	35.5	27.3	8.2	2.67	2.90	92.1	2.84
12 days	35.0	26.2	8.8	2.86	3.22	88.8	2.27
14 days	34.8	25.6	9.2	2.99	3.20	93.4	1.87
800-MC	34.8	25.4	9.4	3.06	3.61	84.8	0.35
389-19A	15.8	4.45	11.35	3.69	5.26	70.1	2.25

[a] Separate flasks.

Earlier survey analyses with aqueous bromine buffered at pH 3.0 resulted in apparent purities exceeding 100%. These high values were usually associated with fermentations that showed much pigmentation and low acid production. Though no purity index above 100 was encountered when the analysis was conducted at pH 1.2, unsaturated pigments should be considered as possible interfering substances in evaluating the results of the bromine absorption method.

LITERATURE CITED

(1) Calam, C. T., Oxford, A. E., and Raistrick, H., *Biochem. J.*, 33, 1488 (1939).
(2) Clark, W. M., and Lubs, H. A., *J. Biol. Chem.*, 25, 479 (1916).
(3) Koppeschaar, W. F., *Z. anal. Chem.*, 15, 233 (1876).
(4) Linstead, R. P., and Mann, J. T. W., *J. Chem. Soc.*, **1931**, 723.

Assessment of Hydrolyzate Solutions for Nutrition

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THE need of an animal for a particular food substance is frequently indicated by its willingness to eat the material. Sometimes the amount of food eaten voluntarily may be used in the measurement of its efficacy, even though appetite per se is unreliable as an exact index of need (*2*)—for example, the ability of rats to determine the presence of tryptophane in the diet, as indicated by an increase or decrease in food consumption, has often been noted (*1*).

In making solutions of protein hydrolyzates, it became necessary to know whether processing had deleteriously affected their nutritive value. It was believed that if the solution were evaporated on a nitrogen-free diet, certain constituents might be harmfully affected from a nutritional standpoint. The simplest alternative was to offer the solution to rats as their sole source of nitrogen and determine whether sufficient solution would be ingested for rapid growth. The hydrolyzates incorporated into the basal diet at levels of 1.2, 1.7, and 2.4% nitrogen are originally made in powder form. Therefore they can be mixed directly with the basal diet. The only hydrolyzate evaporated on the basal diet was one of the two purchased on the open market (Table I).

Accordingly, young rats were supplied a basal dry diet containing all growth essentials save protein; and water and the hydrolyzate solution were offered in separate drinking tubes. Among ten rats there would usually be one or two which would not drink the solutions, and did not grow, even though others in the group showed good gains. This unwillingness of an occasional rat to drink the solution makes the average growth figures subject to considerable variation.

The more dependable assessment of growth can be made by incorporating the dry hydrolyzate in the diet. By comparing this growth with the average growth when a solution of the same material was drunk, an estimate of the usefulness of this procedure can be made. Dry preparations are not always available for assay, and perhaps some information concerning the nutritive value of a solution may be obtained by the procedure.

EXPERIMENTAL

Groups of ten rats, 21 to 23 days of age and weighing about 50 grams, equally distributed as to sex and litter, were placed in individual wire-screen cages, and given a diet of the following composition: dextrin, 82; lard, 9; salt mixture (*2*), 4; cod liver oil, 2; wheat germ oil, 1; brewer's yeast, 2; thiamine, 0.0006; riboflavin, 0.0002%. Water and the hydrolyzate solution were offered by means of two drinking tubes attached to the side of the cage. The volumes consumed daily were noted.

The tubes which contained the 10% hydrolyzate solution were cleaned and refilled every other day. To minimize bacterial growth, 0.5% of sodium benzoate was added. This preservative is more effective than 5% ethyl alcohol and has no deleterious effect on the animal, nor does it affect the taste of the solution. The rats were weighed weekly for four weeks.

In order to compare the average growth with the results obtained by the usual technique, the same lots of protein hydrolyzate were incorporated in the basal diet and fed to groups of ten rats each. Three levels of intake were employed, equivalent approximately to 10, 14, and 20% of the dried hydrolyzate but calculated to supply 1.2, 1.7, or 2.4% of nitrogen, respectively. The average gain in weight for a period of four weeks was determined.

The data are presented in two tables, similarly arranged, and comparison is made between the average growth of the animals receiving the solution and those fed the hydrolyzate in the diet. Growth on the solution was not so consistent as growth on the material incorporated in the diet at a fixed percentage, but if only a qualitative comparison is made, it is obvious that when the hydrolyzate in the diet promoted growth, the same hydrolyzate in solution was also effective.

In Table I, the assays under preparation 403 show that considerable variation was observed when the solution was offered to different groups of rats. Of the six assays, four were reasonably consistent with each other and two gave results considerably higher and lower than these four. When the dry preparation 38 was incorporated in the diet and fed to six groups of rats, five of the assay groups were consistent and only one gave a slightly different value.

This was true also with the five trials of preparation 402 incorporated in the diet. The other values in the table likewise indicate that the solution assay is a much less exact procedure than the usual one of incorporating a fixed amount of the material in the diet.

Table II presents data on single assays of various hydrolyzates given by drinking tube, and assays of the same material incor-

Table I. Average Gain per Rat per Day for Groups of Not Less Than 10 Rats Supplied Protein Hydrolyzates[a] in Solution or in Diet

Preparation	10% Solution	Nitrogen Level in Diet 1.2%	1.7%	2.4%	Average Gain 10% soln.	1.2% N	1.7% N	2.4% N
	Grams gain per day				*Grams per gram of nitrogen ingested*			
403	1.22	1.31	2.07	2.55	12.8	14.9	12.8	11.8
	1.39			2.24	13.2			11.4
	1.86			2.68	16.8			13.5
	1.91				16.5			
	2.13				17.3			
	3.30				16.6			
38	2.49		2.77	3.00	16.4			
			2.37				14.4	
			2.11				15.6	
			1.80				13.8	
			2.03				15.0	
			2.00				14.3	
402	2.64	1.85	2.60	3.04	15.0	17.1	19.0	12.7
				2.39				11.9
				3.24				14.3
				3.09				12.1
				3.15				13.4
10,012	1.71	1.39	2.25		16.4	12.8	15.6	
	1.25	1.17	2.24		16.4	12.6	14.5	
10,014	2.21	1.50	2.08		16.4	14.4	13.6	
	1.09	1.52			12.3	15.1		
40	−0.06		0.81	1.44			9.1	8.5
			0.85				8.0	
415	0.67	0.73	1.68	2.63	7.9	11.8	15.9	13.6
	1.18				10.4			
Preparation A	−0.26	−0.14						
Preparation B	0.33		1.59		7.45		12.9	
Acid-hydrolyzed casein	−0.60		−0.31					
			−0.50					

[a] Code numbers in both Tables I and II indicate experimental lots of Amigen (a pancreatic hydrolyzate of casein) made during the development of the product. Because of differences in preparation, some lots promoted better growth than others. Preparations A and B were purchased solutions of casein hydrolyzates which were fed by tube and also evaporated on the basal diet. The acid-hydrolyzed casein was made by boiling with sulfuric acid in the usual way.

Table II. Average Gain per Rat per Day for Groups of 10 Rats Supplied Protein Hydrolyzates in Solution and in Diet

Preparation	10% Solution	Nitrogen Level in Diet 1.2%	1.7%	Average Gain 10% soln.	1.2% N	1.7% N
	Grams gain per day			Grams per gram of nitrogen ingested		
10,001	2.40	1.92	2.68	16.5	17.3	17.6
10,002	1.32	1.56	2.46	11.2	16.4	16.3
10,003	1.93	2.08	3.49	14.8	17.8	18.8
10,004	1.36	1.93	3.24	12.9	16.5	17.9
10,005	2.54	1.90	2.68	14.4	17.4	17.3
10,006	1.23	1.64	2.83	14.7	17.5	17.5
10,007	0.86	1.80	2.86	10.5	15.1	17.3
10,008	1.08	1.66	3.04	12.6	17.0	19.1
10,009	1.05	1.65	2.49	10.7	17.7	17.3
10,010	1.34	1.95	2.80	11.2	19.1	17.3
10,011	0.90	1.66	2.75	12.8	16.1	15.9
10,013	2.12	1.55	2.46	17.5	15.1	15.9

porated in the diet at two intake levels. In no case did the average growth of the rats receiving the 10% solution equal the growth when 14% of the hydrolyzate (1.7% nitrogen) was incorporated in the diet. Only in three instances was there close approximation in growth (10,001, 10,005, and 10,013) and this can only be taken to indicate that all the rats in these particular groups drank the offered solution in good amount. In general the average gain in the solution group approximated that of 10% (1.2% nitrogen) of the hydrolyzate, since the average growth of all the solution groups was 1.51 grams and of the groups fed 1.2% nitrogen was 1.78 grams. However, the individual groups fed 1.2% nitrogen varied from −75% to +209% of the gain on the corresponding solutions, so that the variability in the results precludes a single assay of a solution from being conclusive.

CONCLUSION

When solutions of hydrolyzates are offered to rats as the sole source of dietary nitrogen, a rough approximation of their value for growth can be obtained. The accuracy of the procedure cannot be compared with that of the classic methods for the assessment of biological value of proteins. The procedure may be helpful when dry preparations are not available for standard biological assay.

LITERATURE CITED

(1) Cox, W. M., Jr., and Mueller, A. J., *Proc. Soc. Expt. Biol. Med.*, 42, 658–63 (1939).
(2) *Nutrition Revs.*, 2, 199 (1944).

Electrogravimetric Determination of Copper in Copper-Base and Tin-Base Alloys

By Controlled Potential Electrolysis

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IN A previous paper (*4*) a relatively simple apparatus was described which automatically controls the potential of an electrode at any desired constant value during the entire course of an electrolysis, and thus renders the application of "graded potential" procedures (*5*) as convenient as the much less selective "constant current" methods. The present paper demonstrates the utility of this apparatus for the direct determination of copper in the presence of tin, antimony, lead, and various other metals, and presents a procedure for determining copper in tin-base and copper-base alloys that requires no preliminary separations.

In the procedure described herein the copper is deposited from a slightly acid tartrate solution, which, for the separation of copper from tin, possesses a number of advantages over the hydrochloric acid solution used in the well-known Schoch-Brown method (*1*, *6*, *7*); although Schoch and Brown used tartrate to separate copper from antimony, it has not previously been used to separate copper from tin. From an acidic tartrate solution cupric copper is reduced directly to the metal, whereas reduction from a hydrochloric acid solution is complicated by stepwise reduction through the cuprous state. Stannic tin forms a much more stable complex ion with tartrate ion than with chloride ion; indeed, the stannic tartrate complex is so stable that no reduction wave is observed with the dropping mercury electrode with stannic tin in acidic, neutral, or basic tartrate solutions (*2*). A few centigrams of copper can be determined accurately in the presence of as much as 2 grams of tin by the procedure described below. Furthermore, both antimonous and antimonic antimony form sufficiently stable complex ions with tartrate ion to permit the determination of copper in the presence of antimony without difficulty, whereas antimony codeposits more or less completely with copper from a hydrochloric acid solution. With the exception of bismuth, the other metals commonly present in tin-base and copper-base alloys also form more or less stable tartrate complexes, which fact effectively prevents their interfering with the determination of copper.

APPARATUS

A cylindrical platinum gauze cathode 5 cm. high and 5 cm. in diameter was used. A platinum gauze cylinder 5 cm. high and 2.5 cm. in diameter was employed as anode, and it was mounted inside and coaxially with the cathode. Efficient stirring was provided by a motor-driven glass stirrer whose shaft passed down through the center of the anode cylinder. The blades of the stirrer were in the form of a large U, whose arms projected well up into the annular space between the electrodes. A saturated calomel electrode was used to control the cathode potential. A 6-mm. tube filled with a 3% agar gel in saturated potassium chloride served as a salt bridge, and its tip was placed outside and as close as possible to the cathode cylinder at about its middle. An ordinary 250-cc. beaker served as the electrolysis cell. A thick (1.25-cm.) piece of Bakelite plate, with clamps for the electrode leads and holes for the stirrer shaft and salt bridge, was used as a cover.

The electrical circuit, by means of which the potential of the cathode was controlled automatically to within ±0.02 volt at any desired value, has been described in detail (*4*). This apparatus functioned very satisfactorily without attention during the entire course of an electrolysis.

PROCEDURE

A 0.5- to 2-gram sample of the alloy was weighed into a covered 250-cc. beaker and dissolved in a warm mixture of 8 cc. of 12 *N* hydrochloric acid and 2 cc. of 16 *N* nitric acid. The nitric acid was added in several small portions as needed. The solution was boiled very gently for a minute or two to remove most of the oxides of nitrogen and chlorine. Then 100 cc. of a solution containing 23 grams (0.10 mole) of reagent quality sodium tartrate dihydrate, 1 gram of urea to remove oxides of nitrogen, and 10 cc. of 5 *N* sodium hydroxide, were added. The solution was diluted to about 200 cc., treated with 1 to 2 grams of hydroxylamine

hydrochloride as an anodic depolarizer, and electrolyzed with a cathode potential of −0.36 volt *vs.* the saturated calomel electrode.

The solution of the alloy cannot be diluted with water and treated with solid sodium tartrate, because when much tin is present the hydrous stannic oxide which precipitates on dilution with water dissolves only very slowly after the tartrate is added. When the solution is composited as above the tin remains completely in solution as a tartrate complex.

Lead hydrogen tartrate is only moderately soluble in a tartrate solution of the above composition, and hence when much of this element is present it may precipitate as coarse white crystals after the addition of the tartrate solution. In such a case the solution should be filtered before finally diluting to 200 cc. Under the conditions given above as much as about 50 mg. of lead may be present without precipitation.

The amounts of acid, sodium hydroxide, sodium tartrate, and hydroxylamine hydrochloride specified above yield a solution that contains tartrate and hydrogen tartrate ions in about equimolar amounts, and has a pH value in the neighborhood of 4 to 4.5. This composition was chosen because experience has shown that it is optimum for the separation of copper from various metals with a mercury cathode (*3*).

In an acidic tartrate solution of the above composition the polarographic half-wave potential of copper is −0.09 volt, that of bismuth is −0.29 volt, and that of lead is −0.48 volt *vs.* the saturated calomel electrode (*2*, *3*). Tin, antimony, and the other metals commonly present in tin-base and copper-base alloys all have more negative reduction potentials. In agreement with expectations based on these polarographic half-wave potentials it was found that the optimum cathode potential for the determination of copper in the presence of lead, tin, antimony, and the other metals commonly present in tin-base and copper-base alloys is in the range from −0.30 to −0.40 volt *vs.* the saturated calomel electrode. Lead deposits when the potential is more negative than about −0.40 volt, and if the potential is much less negative than about −0.30 volt the rate of deposition of copper is slow. Most of the determinations were run with the cathode potential at −0.36 ± 0.02 volt *vs.* the saturated calomel electrode. An electrolysis time of 60 minutes was adequate in most cases (see Figure 1).

The electrolysis was stopped by lowering the beaker away from the electrodes without disconnecting the circuit. The cathode was then washed quickly with water, dipped into two baths of pure acetone, dried for 3 minutes in an oven at 70° C., and weighed after cooling in the air for at least 20 minutes. The copper deposits invariably displayed excellent characteristics, being almost mirror-bright and of a salmon pink color.

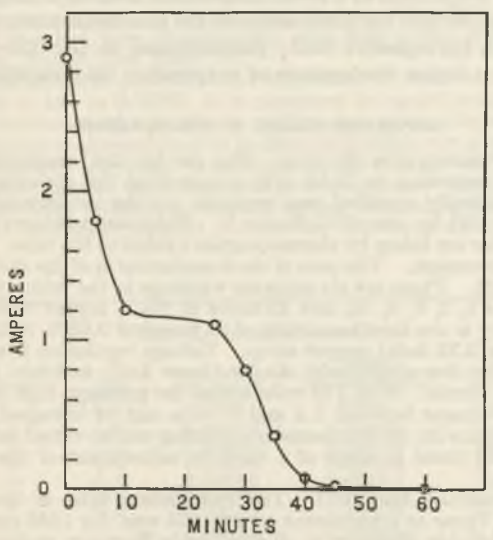

Figure 1. Typical Current-Time Curve

Table I. Determination of Copper in Bureau of Standards Copper-Base and Tin-Base Alloys

Alloy	Copper Found, %
Ounce metal 124a Cu 85.05, Pb, 4.86, Sn 4.81, Zn 5.25%	85.02 84.94 84.99 84.99 85.06 Av. 85.00 ±0.03
Phosphor bronze 63a Cu 78.48, Sn 9.76, Pb 8.92, Sb 0.49, Zn 0.61, P 0.58, Fe 0.52, Ni 0.32, S 0.11	78.47 (78.32)[a] 78.54 78.47 78.46 Av. 78.49 ±0.03
Tin-base bearing metal 54b Cu 3.19, Sn 87.45, Sb 7.39, Pb 1.81, Bi 0.027, Ag 0.030, As 0.051, Fe 0.029	3.17[b] 3.18 3.18 Av. 3.18

[a] Omitted from average.
[b] Corrected for bismuth and silver.

RESULTS AND DISCUSSION

All the results obtained with three Bureau of Standards alloys are shown in Table I. One-gram samples of the ounce metal, 0.5-gram samples of the phosphor bronze, and 2-gram samples of the tin-base bearing metal were used, and perfectly clear solutions were obtained without filtration.

One of the advantages of electrogravimetric determinations at controlled potential is that the current serves as a reliable criterion of the progress of electrolysis (*3*, *5*). This is demonstrated by the typical current-time curve in Figure 1, obtained during the electrolysis of one of the ounce metal samples at a constant cathode potential of −0.36 volt. Following a rapid decrease from an initial value of 2.9 amperes, the current remained roughly constant during the middle stage of the electrolysis, and then decreased again and approached zero asymptotically. After 45 minutes the current had decreased to 0.025 ampere, and it finally fell to 0.008 ampere after 60 minutes, at which time the electrolysis was discontinued. In all the determinations listed in Table I the current decreased to less than 0.02 ampere after 60 minutes.

It is evident that the present simple procedure yields results that are as precise and accurate as those obtained by the much longer methods on which the bureau's values are based. The results obtained with the tin-base bearing metal are particularly significant, since this sample constitutes about as unfavorable a case as is likely to be encountered. The small amounts of silver and bismuth present in the tin-base alloy codeposited with the copper and the appropriate correction has been applied.

Relatively large amounts of tin, antimony, lead, and zinc, and small amounts of iron, nickel, arsenic, and phosphorus do not interfere. There is no evident reason why the method will not be equally successful in the presence of much larger amounts of antimony, iron, and nickel than were present in these samples. Obviously, the method will be equally satisfactory for simple brasses and bronzes, which present no problems not anticipated by the alloys studied, and it should also be applicable in the presence of such elements as manganese, aluminum, chromium, cobalt, vanadium, uranium, cadmium, and others whose reduction potentials are more negative than that of copper. Bismuth, gold, mercury, and the metals of the platinum group will interfere. More than traces of silver will precipitate as silver chloride during the solution of the sample, and can be removed by filtration before electrolysis.

Since it was shown in a previous study (*3*) that with a mercury

cathode at controlled potential copper can be separated from bismuth in an acidic tartrate solution, numerous attempts were made to effect this separation with the platinum cathode. Even though the cathode potential was kept as low as −0.12 volt, which is lower than is necessary with the mercury cathode, and the pH of the tartrate solution was varied from about 3 to 6, it was not possible to achieve a separation with the platinum cathode. It was observed from the color of the deposit in these experiments that copper alone deposited during the initial stages of the electrolysis, but when the deposition of copper was almost complete deposition of bismuth suddenly commenced and proceeded to completion.

Many attempts were made to determine lead in the solutions remaining from the copper determinations. The copper-plated cathode was replaced in the solution and the lead was deposited as the metal with the potential of the cathode at various values between −0.56 and −0.80 volt *vs.* the saturated calomel electrode. Although particular care was taken to wash the deposited lead very quickly with distilled water and acetone, the results were unsatisfactorily low in every case. The average percentage of lead found in the tin-base alloy was 1.77 ± 0.05 compared with the certificate value 1.81, in the phosphor bronze alloy 8.64 ± 0.09 compared with the certificate value 8.92, and in the ounce metal 4.71 ± 0.08 compared with the certificate value 4.86. These results correspond to a loss of between 0.8 and 1.5 mg. of lead in the various determinations. The low results were not due to incomplete deposition of the lead, because polarographic analyses of the residual solutions showed only a trace of lead that corresponded to only a small fraction of the negative error. By weighing the anode it was established that no lead dioxide deposited. Thus it is fairly certain that the low results were due to loss of lead by partial re-solution of the deposit during the washing, and since such loss cannot easily be avoided this method of determining lead cannot be recommended for anything but approximate analyses.

SUMMARY

A rapid and accurate method is described for determining copper in copper-base and tin-base alloys, based on its deposition at a controlled potential from an acidic tartrate solution. No preliminary separations are required. An apparatus was used which controlled the cathode potential automatically, and determinations were completed in about an hour. Analyses of several Bureau of Standards alloys demonstrated that the method is as accurate as classical electrogravimetric procedures, and that it can be applied in the presence of relatively large amounts of tin, antimony, lead, zinc, and various other metals commonly present in tin-base and copper-base alloys.

LITERATURE CITED

(1) Engelenburg, A. J., *Z. anal. Chem.*, 62, 257 (1923).
(2) Lingane, J. J., IND. ENG. CHEM., ANAL. ED., 15, 583 (1943).
(3) *Ibid.*, 16, 147 (1944).
(4) *Ibid.*, 17, 332 (1945).
(5) Sand, H. J. S., "Electrochemistry and Electrochemical Analysis", Vol. II, London and Glasgow, Blackie and Son, 1940.
(6) Schoch, E. P., and Brown, D. J., *J. Am. Chem. Soc.*, 38, 1660 (1916).
(7) Torrance, S., *Analyst*, 62, 719 (1937).

Determination of Small Amounts of Sulfur in Naphthalene

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An analytical method described for the determination of sulfur in naphthalene is applicable to the sulfur range of 0.002 to 2%. This method involves the combustion of naphthalene in a current of purified air and the turbidimetric determination of sulfur as barium sulfate. It is applicable not only to naphthalene but to other high-boiling organic materials. Incidentally, a variable, high-current, low-voltage transformer, a gas-lift absorber of small holdup, and an infrared technique for evaporating analytical samples are described.

THERE has been no satisfactory method available for the determination of small amounts of sulfur in naphthalene, although numerous procedures (*1*, *3*, *4*, *5*, *7*, *8*, *10*, *12*, *14–17*, *20*) are applicable to naphthalene with relatively high sulfur content. The evaluation of naphthalene for catalytic hydrogenation over sulfur-sensitive catalysts requires an analytical method capable of determining sulfur of the order of a few thousandths of a per cent. The excellent method of Zahn (*18*) is not usable because naphthalene cannot be burned in a lamp owing to the deposition of naphthalene on the cooler portions of the wick and the difficulty in obtaining a sootless flame.

The method described here involves the combustion of naphthalene in a hot tube in a current of purified air and the turbidimetric determination of sulfur as barium sulfate. This method has been checked against samples of naphthalene containing known amounts of sulfur and found to give satisfactory results in the sulfur range of 0.002 to 2%. Depending on the sulfur content, the optimum size of sample varies from 1.5 to 0.5 gram. In order to obtain accurate analytical figures in the lower sulfur range, the air-reagent sulfur blank must be of the order of 0.00001 gram and the rate of combustion must be carefully controlled to prevent explosion and obtain complete combustion.

The procedure is specific for naphthalene, but it has also been applied to the analysis of phenols, anthracene, phenanthrene, and alkylated polycyclics. The only modification needed concerns the rate of evaporation of the sample. For example, phenol must be evaporated at a lower temperature because of its greater volatility, so that the composition of the phenol-air mixture does not reach the explosive limit; phenanthrene, on the other hand, requires a higher temperature of evaporation than naphthalene.

APPARATUS (FIGURE 1) AND REAGENTS

AIR-PURIFICATION SYSTEM. The air for the combustion is purified according to Zahn (*18*) except that the stainless steel tube originally specified was replaced on the recommendation of Zahn (*19*) by one of Nichrome V. Tube temperatures of the air purifier are taken by thermocouples welded to the tube.

TRANSFORMER. The core of the transformer is of the shell type (Figure 2). There are six separate windings in the primary, consisting of 1, 2, 4, 8, 16, and 32 turns of No. 6 copper wire; the secondary is one turn consisting of 32 pieces of 0.0375 × 5.6 cm. (0.015 × 2.25 inch) copper strap. Voltage regulation is accomplished by five single-pole, double-throw knife switches in the primary circuit. With 110 volts across the primary, high amperage at voltages between 1.5 and 3 volts can be obtained in 31 steps (Figure 3), for the secondary winding can be varied between 32 and 63 turns in steps of 1 turn by adjustment of the knife switches.

COMBUSTION ASSEMBLY. The combustion tube is made of No. 790 Vycor or transparent quartz, 925 mm. (or 1140 mm.) × 25 mm. outside diameter × 19 mm. inside diameter, sealed to an exit end, 200 mm. long × 10.5 mm. outside diameter, whose

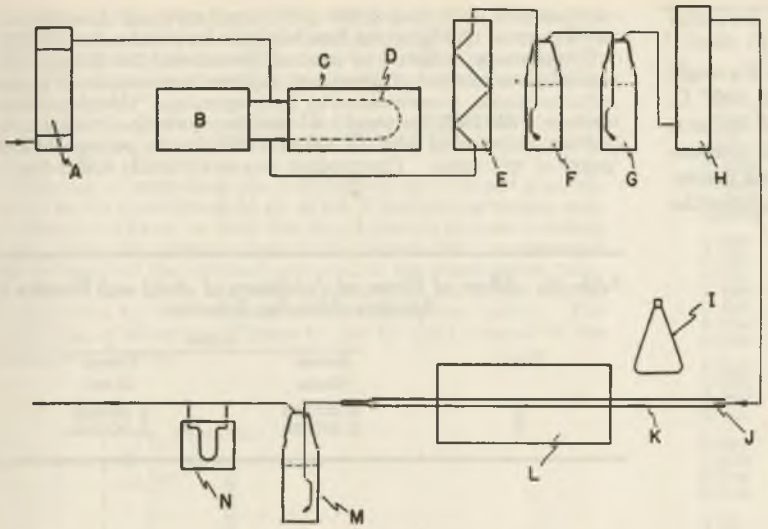

Figure 1. Purification—Combustion—Absorption Assembly

A. Charcoal–glass wool tower	*H.* Glass wool tower
B. Transformer	*I.* Infrared lamp
C. Furnace	*J.* Combustion tube
D. Stainless steel tube	*K.* Combustion boat
E. Water cooler	*L.* Combustion furnace
F. Sodium hypobromite scrubber	*M.* Sodium carbonate absorber
G. Sodium hydroxide scrubber	*N.* Flowmeter

extreme tip is drawn down to 6 mm. outside diameter. The combustion tube is heated at 1100° C., by a single 30-cm. (12-inch) furnace (Fisher No. 10-469), or at 900° C. by two 30-cm. (12-inch) furnaces (Fisher No. 10-472), the longer tube being used in the double furnace assembly. In either case, about 12 inches (30.5 cm.) of tube extend outside of the heated zone at the inlet end.

Absorption Apparatus. The type of absorption apparatus depends upon which of the two equally satisfactory methods of combustion is employed. One method is to aspirate air through the equipment by means of a throttled vacuum at the exit end of the system; the other method is to force compressed air through the equipment via the air purifier. Either fritted-glass absorbers (Pyrex No. 31760, 500-cc.) or gas-lift absorbers (Figure 4) are used if the combustion air is aspirated; gas-lift absorbers are used both in the purification train and for the absorption of the combustion products if the combustion air is forced through the equipment because the fritted-glass plates exert too much back pressure which causes leaks. The preferred method is to force the combustion air through the equipment, thereby avoiding error due to inward leakage of unpurified air. However, it is more convenient to aspirate the combustion air through the apparatus, and with proper care in making the connections, there is little danger of inward leakage.

Turbidimeter. A Hellige turbidimeter (No. 8000-DN) was used that had been standardized according to the directions of Sheen, Kahler, and Ross (*13*).

Reagent and Air-Reagent Blanks. In order to determine sulfur as low as 0.002%, it is necessary to regard every reagent and operation with suspicion. Often several bottles of sodium carbonate or glycerol have to be discarded before one is found with a sufficiently low blank to be made up into a stock solution. For instance, blanks on 200-cc. samples of ordinary distilled water varied from 0.00002 to 0.00005 gram, whereas triple-distilled water (ordinary distilled water that had been distilled twice more, the first time from 0.001 *N* potassium permanganate) gave consistent blanks of 0.00001 gram of sulfur per 200 cc. The accepted procedure is to prepare triple-distilled water in small batches in an all-glass equipment, storing the distillate in 1-gallon (3.79-liter) Pyrex glass-stoppered (paraffin-sealed) bottles and testing each gallon turbidimetrically before acceptance. These extreme precautions are perhaps not always necessary, but it was only after observing them that occasional erratic results were eliminated. Wash bottles should be freshly filled each day, and it is considered advisable to blow into the bottles through a cotton filter.

Table I lists a number of typical air-reagent blanks, obtained by carrying out all the operations of the analytical procedure in the usual manner with the exception that the combustion boat was empty. In the absence of the Zahn air purifier, the air blank was about 0.00002 gram of sulfur per hour (Table I).

Relation between Length and Temperature of Combustion Zone. Originally a single 12-inch furnace at 900° C. was used, but the analytical figures were consistently low—e.g., about 70% of the theoretical for naphthalene samples containing 0.02% of sulfur (Table II).

A 6-inch (15-cm.) roll of platinum gauze did not increase the amount of sulfur found. Although a packing of quartz in the combustion tube might have made the combustion more efficient, it is impossible to use this expedient because it is necessary to

Table I. Air-Reagent Blanks

Hours	Air	Sulfur: Air blank	Sulfur: Reagent blank	Sulfur: Total blank
	Cu. ft.	*Gram*	*Gram*	*Gram*
1	1.2	0.000005	0.000005	0.000010
1	1.2	0.000005	0.000005	0.000010
2	2.4	0.000003	0.000008	0.000011
2	2.4	0.000003	0.000010	0.000013
5	6.0	0.000003	0.000009	0.000012
5	6.0	0.000002	0.000010	0.000012
	Av.	0.000003	0.000008	0.000011

Table II. Analytical Data for Single Furnace at 900° C.

Sample	Sulfur: Actual	Sulfur: Found	Sulfur: Actual	Sulfur: Found	Efficiency
Grams	*Gram*	*Gram*	%	%	%
1.3650	0.000383	0.000271	0.028	0.019	68
1.4698	0.000383	0.000272	0.026	0.017	66
2.1327	0.000383	0.000302	0.018	0.014	78
1.9034	0.000383	0.000297	0.020	0.015	75
2.2553	0.000383	0.000300	0.017	0.013	77

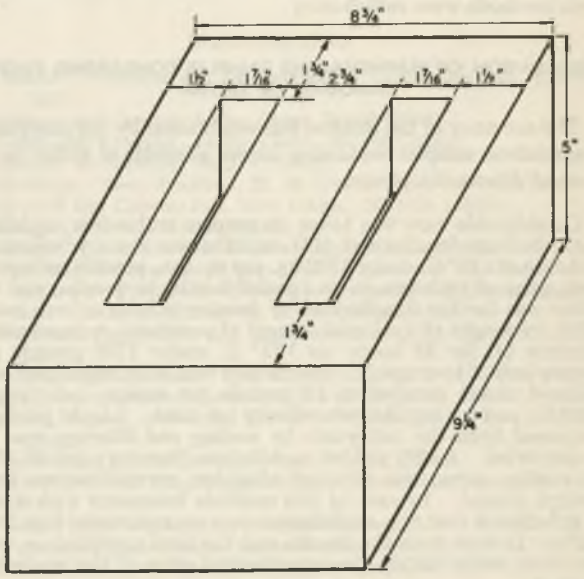

Figure 2. Transformer Core

rinse the tube after combustion (lack of rinsing lowers the sulfur figure by 0.001 to 0.003%).

The substitution of two 12-inch furnaces at 900° C. or of a single 12-inch furnace at 1100° C. for the single furnace at 900° C. raised the amount of sulfur found to the theoretical amount. A single furnace at 900° C. would probably have been satisfactory at a slower combustion rate, but it was considered preferable to keep the time of analysis at a minimum and to raise the efficiency of combustion by using a higher temperature.

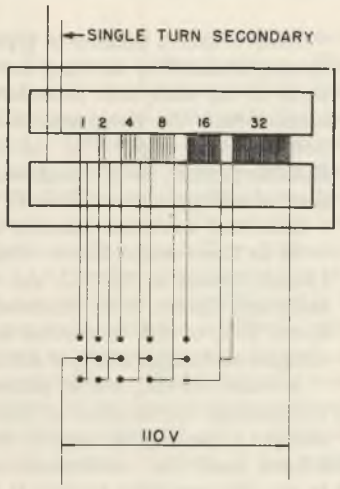

Figure 3. Transformer Wiring Diagram

It was first thought that the low sulfur figures were due to faulty working up of the absorber solution, inasmuch as the solution was acidified while sulfite could still be present. Experiment, however, showed that this explanation was untenable. Two identical absorber solutions were prepared, each containing 0.00035 gram of sulfur (56% as sodium sulfate, 44% as sodium sulfite) in the standard volume of sodium carbonate solution. Each was analyzed turbidimetrically, one (A) being acidified prior to the addition of bromine water, the other (B) being acidified after bromine water had been added. Table III shows that both methods were satisfactory.

PREPARATION OF NAPHTHALENE SAMPLES CONTAINING KNOWN AMOUNTS OF SULFUR

The accuracy of the method was established by the analysis of naphthalene samples containing known amounts of sulfur in the form of dibenzothiophene.

Considerable care was taken to prepare sulfur-free naphthalene. Sodium-desulfurized (*11*) naphthalene was hydrogenated (8 hours at 170° C. under 1700 kg. per sq. cm., pounds per square inch, gage of hydrogen in an Ipatieff bomb) to decalin, and the latter was further desulfurized by heating it with a fresh batch (25% by weight of the liquid charge) of powdered, reduced nickel catalyst (*6*) for 28 hours (at 170° C. under 1700 pounds per square inch of hydrogen). The decalin was dehydrogenated over pelleted nickel catalyst at 15 pounds per square inch (gage), 350° C., and 0.5 liquid space velocity per hour. Liquid product, recovered from the catalyzate by cooling and filtering, was recycled twice. A 50% yield of naphthalene (freezing point 80.2° C. by cooling curve) was obtained after two crystallizations from methyl alcohol. In view of this multiple treatment with nickel it is believed that the naphthalene was exceptionally free from sulfur. In fact, both the decalin and the final naphthalene were free from sulfur within the experimental error of the analytical method.

Dibenzothiophene was prepared according to the direction of Gilman and Jacoby (*2*). After crystallizing three times from ethyl alcohol, it melted at 99–100° (capillary) and showed a sulfur content of 17.3% by the Parr bomb (theoretical sulfur, 17.4%).

Cyclohexane, which was used as the solvent for dibenzothiophene in one method of preparing naphthalene samples of known sulfur contents, was obtained by hydrogenating "thiophene-free" benzene with 25% by weight of powdered, reduced nickel catalyst for 24 hours at 150° C. under 1700 pounds per square inch (gage) of hydrogen. The product was analytically sulfur-free.

Table III. Effect of Order of Additions of Acid and Bromine to Alkaline Absorber Solution

Expt.	Sulfur	
	Actual	Found
	Gram	*Gram*
A	0.000350	0.000343
B	0.000350	0.000340

Naphthalene-dibenzothiophene samples were prepared by two methods. One method was to add a known amount of dibenzothiophene dissolved in 1 cc. of cyclohexane to a weighed sample of sulfur-free naphthalene in a combustion boat. The cyclohexane was allowed to evaporate for one hour before combustion of the sample in order to avoid explosion during combustion. Three cyclohexane solutions were used to obtain sulfur contents at the three desired levels of 0.2, 0.02, and 0.002%. The second method, which was used for the higher sulfur concentrations, was to weigh the required amounts of naphthalene and dibenzothiophene directly into the combustion boat. Subsequently it was shown that true samples could be prepared by mixing known weights of the two components in liquid condition (at 100° C.) in a sealed tube and quickly freezing the mixture. This method was not used in the actual calibration of the analytical method because there was a suspicion that segregation might take place during crystallization.

Amount of Air Used and Danger of Explosion. With 0.5- and 1.5-gram samples, which are the weights recommended for 0.2 and 0.002% of sulfur, respectively, the volume of combustion air (550 cc. per minute, 0.02 cu. foot per minute) used is six times and two times, respectively, the theoretical amount required for complete combustion. Assuming constant evaporation rate through the hour required for the combustion, the naphthalene content of the gas stream is considerably below the explosive limit (*9*) in the case of a 0.5-gram sample of naphthalene but close to the limit with a 1.5-gram sample. The authors have never experienced a violent explosion in 300 analyses. If the sample is evaporated too rapidly, explosion waves are set up which oscillate gently back and forth through the heated zone, with little or no carbon formation, the accuracy of the analysis being usually unaffected. Even these gentle explosions are rare with an experienced operator. Nevertheless, in anticipation of the unexpected, it is recommended that the combustion-absorption assembly be shielded by a shatterproof glass screen and that the analyst wear goggles, at least until familiar with the method, and, even when experienced, that these precautions be observed whenever an unfamiliar sample is being burned.

ANALYTICAL PROCEDURE

Combustion. The combustion furnace and air purifier are brought up to temperature. The porcelain boat containing the sample of naphthalene is placed in the combustion tube, 2 inches (5 cm.) outside of the furnace and 8 inches (20 cm.) from the inlet end of the tube. The inlet end of the combustion tube is connected to the air purifier, the exit end is connected to the absorbers, and the flow of combustion air is started (absorption and air flow described below). The sample is melted by a 250-watt infrared lamp placed about 6 inches (15 cm.) above the boat, and the rate of evaporation is controlled by lowering the lamp until, at the end of 45 minutes, it almost touches the combustion tube. The infrared technique is very useful in evaporating analytical samples. When the boat appears dry, the furnace is pushed over

the section of the tube containing the boat. The combustion of a 1.5-gram sample of naphthalene requires one hour. Two combustion-absorption assemblies can be serviced by a single air purifier which makes it possible to run six to eight combustions in an 8-hour day.

ABSORPTION. Sodium carbonate was substituted on the recommendation (*19*) of Zahn for the sodium hypobromite originally specified (*18*).

In the case of aspirating the combustion air, a fritted glass absorption bottle (containing 25 cc. of 0.5 *N* sodium carbonate solution diluted to 150 cc. or until the liquid level is at least 3 inches, 7.5 cm., above the upper surface of the fritted disk) is connected (glass to glass) to the reduced exit end of the combustion tube. The vacuum flow meter assembly is set at 0.02 cu. ft. per minute and connected to the exit end of the absorption bottle. The gas-lift type of absorber (Figure 4) can be used instead of the fritted-glass absorber.

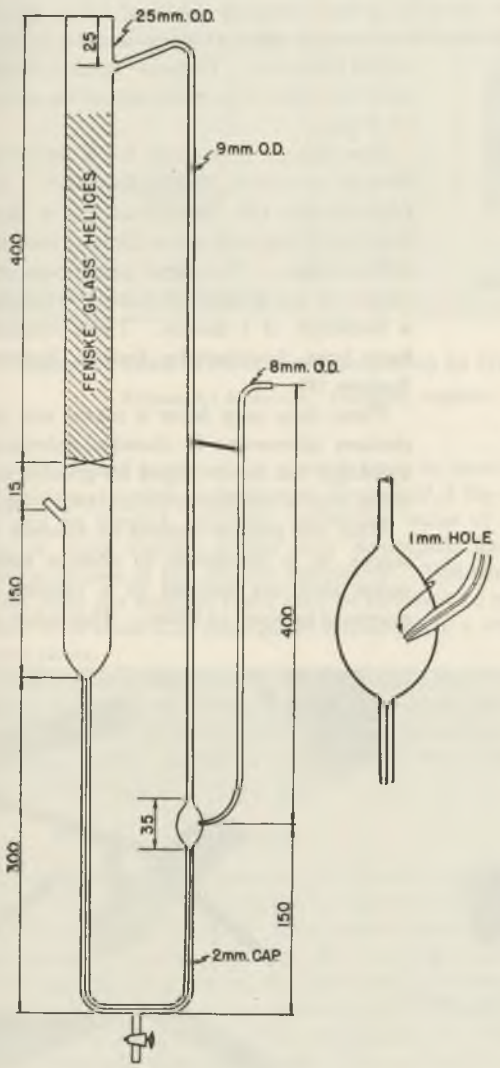

Figure 4. Gas-Lift Absorber

All dimensions in millimeters

If the combustion air is forced through the equipment, the inlet end of the air purifier is connected to a source of compressed air via a pressure regulator, and the rate of air is set at 0.02 cu. foot per minute by a flowmeter attached to the exit end of the absorber.

ANALYSIS OF ABSORBER SOLUTION. The rubber connection between the combustion tube and the absorber is cut and the contents of the absorber are transferred to a 500-cc. Erlenmeyer flask, together with two 20-cc. rinsings. The hot combustion tube is removed from the furnace and placed horizontally on a rack to cool. When it has cooled to room temperature, it is rinsed twice with 20-cc. portions of distilled water which are added to the Erlenmeyer. The solution is made acid to methyl red with 1 *N* hydrochloric acid, 15 cc. of saturated bromine water are added, and the solution is evaporated on a hot plate to about 25 cc. It is then made slightly alkaline to phenolphthalein with 20% sodium hydroxide and exactly neutralized with 1 *N* hydrochloric acid, after which 3 cc. of acid are added. The solution is filtered through a Whatman No. 42 filter paper into a 50-cc. volumetric flask and made up to the mark. The turbidity is determined according to Zahn (*18*) and Sheen, Kahler, and Ross (*13*). If the amount of sulfur in the 50 cc. of solution is greater than 0.00055 gram, the solution is suitably diluted and an aliquot is taken which contains approximately this amount. The sulfur content of the naphthalene is calculated after the sulfur blank has been subtracted.

Table IV. Determination of Sulfur on Samples of Known Composition

Sulfur			
Actual	Found	Difference	Absolute Error
%	%	%	%
0.0024	0.0028	+0.0004	+17
0.0021	0.0027	+0.0006	+29
0.0029	0.0032	+0.0003	+10
0.0024	0.0033	+0.0009	+38
0.027	0.027	0.000	0
0.032	0.031	−0.001	− 3
0.024	0.023	−0.001	− 4
0.026	0.025	−0.001	− 4
0.022	0.020	−0.002	− 9
0.026	0.924	−0.002	− 8
0.025	0.025	0.000	0
0.253	0.236	−0.017	− 7
0.288	0.265	−0.023	− 8
0.262	0.247	−0.015	− 6
0.263	0.243	−0.020	− 8
0.807	0.792	−0.015	− 2
0.538	0.500	−0.038	− 7
0.744	0.708	−0.036	− 5
0.543	0.459	−0.084	−16
2.68	2.32	−0.36	−14
1.69	1.80	+0.11	+ 7
1.73	1.70	−0.03	− 2
1.87	1.56	−0.31	−17

The data for naphthalene samples of known sulfur contents presented in Table IV reveal the order of accuracy which is obtained by this method of analysis. It is believed that the absolute error is not more than 20% except in the range 0.002% of sulfur, the lowest limit to which the method was applied; here, the absolute error is believed to be not more than 50%.

LITERATURE CITED

(1) Eininov, Plakhuta, and Lantsevitskaya, *Zavodskaya Lab.*, **6**, 566 (1937).
(2) Gilman and Jacoby, *J. Org. Chem.*, **3**, 108 (1938).
(3) Grote and Krekeler, *Angew. Chem.*, **46**, 106 (1933).
(4) Heinze, *Braunkohle*, **39**, 519 (1940).
(5) Heslinga, *Chem. Weekblad*, **22**, 98 (1925).
(6) Ipatieff and Corson, IND. ENG. CHEM., **30**, 1039 (1938).
(7) Lange, *Glückauf*, **78**, 650 (1942).
(8) Muller, Hiller, and Klaude, *Brennstoff.-Chem.*, **13**, 145 (1932).
(9) National Fire Protection Association, Committee on Flammable Liquids, "Properties of Certain Flammable Liquids, Gases, and Volatile Solids", p. 35, Boston, Mass., 1935.
(10) Schoberl, Jarczynski, and Rambacher, *Angew. Chem.* **50**, 334 (1937).
(11) Schroeter, U. S. Patent 1,763,410 (1930).
(12) Seuthe, *Arch. Eisenhüttenw.*, **11**, 343 (1938).
(13) Sheen, Kahler, and Ross, IND. ENG. CHEM., ANAL. ED., **7**, 262 (1935).
(14) Sielisch and Sandke, *Angew. Chem.*, **45**, 130 (1932).
(15) Skhodtsev and Blagoveshchenskaya, *Zavodskaya Lab.*, **7**, 88 (1938).
(16) Velikovskaya and Zelikov, *Neftyanoe Khoz.*, **18**, No. 8, 52 (1937).
(17) Wurzschmitt and Zimmermann, *Z. anal. Chem.*, **114**, 321 (1938).
(18) Zahn, Victor, IND. ENG. CHEM., ANAL. ED., **9**, 543 (1937).
(19) Zahn, Victor, private communication.
(20) Zelikov, Kotrelev, and Fogelson, *Neftyanoe Khoz.*, **26**, No. 5, 58 (1934).

A Use of the Electron Microscope in Chemical Microscopy

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In this paper the possibilities that the electron microscope may offer in chemical microscopy are discussed, a technique for adapting the electron microscope to this study is presented, and typical pictures are shown of results obtained with the technique described. The technique for the controlled growth of crystals on thin plastic films is based on the diffusion of ions and molecules through the plastic film and subsequent precipitation on the film.

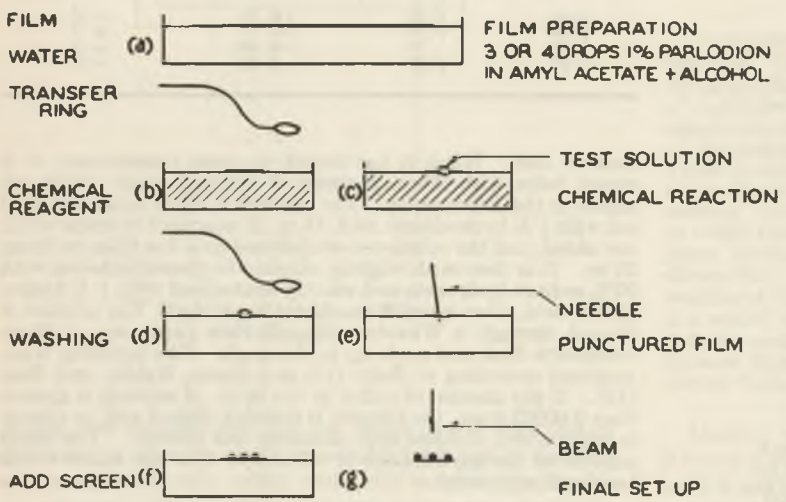

Figure 1. Crystal Preparation

THE light microscope has been used for many years to study the habit of crystals of insoluble precipitates as a means of chemically identifying the material. The first attempt at systematic microscopical qualitative analysis was proposed in 1877 by Boricky (*1*). Subsequent contributions by Behrens, Emich, Donau, and others developed the methods and techniques of chemical microscopical analyses. These methods are described in Chamot and Mason's (*3*) handbook.

In chemical microscopy, the light microscope is used to aid the eye in distinguishing the crystal habit of a substance. This habit is correlated with its chemical composition. The crystals studied are usually prepared by causing a reaction of a solution or crystal of the unknown with test reagents. For some reactions, if reproducible crystal habits are to be obtained, the concentration, pH, temperature, rate of growth, and other factors must be controlled. Additional information which may be helpful in identifying the material can be obtained from polarized light and refractive index studies on the crystal.

During the past decade the electron microscope has been developed to a point where it can be applied to a wide variety of problems. The purpose of this paper is (1) to investigate the possibilities that the electron microscope may offer in chemical microscopy, (2) to present one technique for adapting the electron microscope to this study, and (3) to show typical pictures of some results obtained with the technique described.

THE ELECTRON MICROSCOPE

Three factors in general limit the use of the light microscope: (1) the limited resolving power, (2) the limited depth of focus, and (3) the short working distance. Thus, the observation of detail is limited to an effective magnification of 1200 diameters. However, in order to obtain sufficient depth of focus, most light microscopic observations are made at magnifications of the order of 200 diameters. Therefore special precautions must be taken to grow crystals of the proper size for study.

The electron microscope has a useful magnification of about 50,000 diameters. At this magnification the instrument has a depth of focus of 10 microns and a limit of resolution of 0.004 micron. The light microscope at 200 diameters has a depth of focus of 4 microns and a resolution of 1 micron. These relationships have been described by Burton, Barnes, and Rochow (*2*).

These facts may favor a useful role for the electron microscope in chemical microscopy if technique can be developed for growing crystals which may be studied in the electron microscope.

With the present designs of electron microscopes, it is impossible to observe specimens unless they are exposed to a vacuum of 0.1 micron of mercury or better. This factor, as well

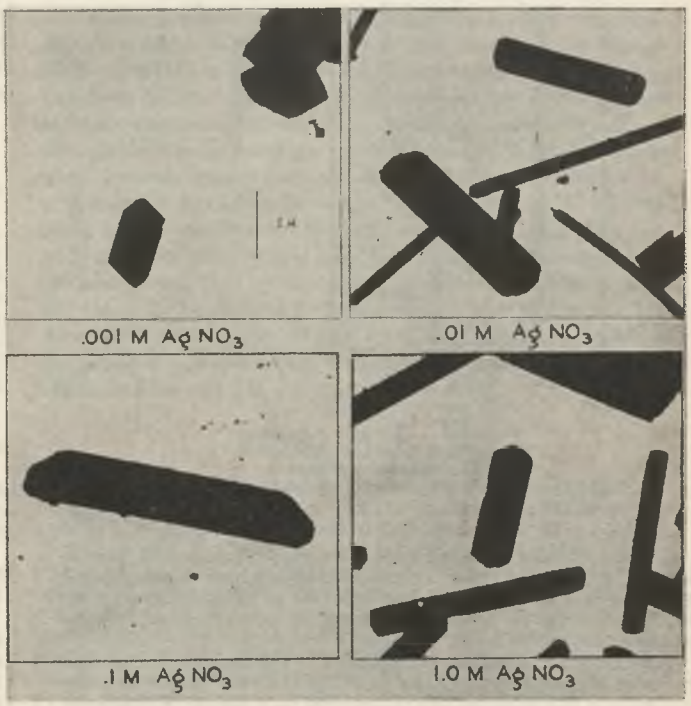

Figure 2. Effect of Concentration of Test Solution on Crystal Form

Reagent, 0.1 *M* K_2CrO_4. Precipitate, Ag_2CrO_4

Figure 3. Effect of Method of Precipitation on Crystal Form

Reagent, 0.1 *M* K_2CrO_4. Precipitate, Ag_2CrO_4

as the absorption of energy of the electron beam by specimens of finite thickness, imposes limitations on the nature of the crystals which may be studied. Crystals containing water of crystallization or other volatile matter may decompose under the vacuum conditions in the electron microscope. The absorption of energy from the electron beam may be sufficient to melt the substance or to cause it to undergo decomposition or a transition of crystal phase.

The shadow technique is used for the study of small crystals, which are supported by a thin plastic film mounted on a fine-mesh metal screen. If the thin plastic film can be used for preparation of the crystals as well as for support in the microscope, the over-all technique will be simplified.

METHOD

The preparation of specimens for the electron microscope requires that the material be evaporated to dryness before being placed in the vacuum chamber. If the crystals are produced by the interaction of the two solutions, they must be collected in a dispersed manner on a thin plastic film and the mother liquor removed. This latter fact is an important difference in the manner of preparation of crystals for the electron microscope as contrasted with the light microscope. Crystals studied in the light microscope in chemical microscopy are usually observed in their mother liquor.

Many methods have been proposed for carrying out tests in chemical microscopy (*3*). Although a few of them could be adapted for use with the electron microscope, it is of considerable advantage to utilize the property of diffusion of ions and molecules through thin plastic films. The technique then is to collect, wash, and observe the crystals on the same piece of plastic film. The method is shown schematically in Figure 1.

A large section of Parlodion film is formed on clean distilled water, using 3 to 4 drops of a 1% solution of Parlodion in a 2 to 1 solvent mixture of amyl acetate and ethyl alcohol. The purpose of the alcohol is to increase the porosity of the film. Sections of the film, 1 cm. in diameter, are removed by raising a small ring under the Parlodion film; a small needle is used to cut the film away from the ring. The sections of film are now placed on the surface of the reagent in a small crystallizing dish. The concentration of the reagent is 0.1 molar. A small drop of the test solution is placed on the upper surface of the film by a small glass capillary tube. Diffusion of the ions through the film allows the formation of an insoluble crystalline precipitate in the drop where solubility factors permit and if test solutions which are known to produce crystalline precipitates are chosen for study. The time necessary to produce sufficient crystals for observation in the electron microscope is of the order of 1 to 2 minutes. A small hand lens is useful in observing the extent of crystallization.

The Parlodion film and crystals are removed from the reagent solution (Figure 1, *c*) by means of a second ring (with handle) slightly smaller in diameter than the one used in Figure 1, *a*. The film is now gently deposited on the surface of clean distilled water in a crystallizing dish. The water and ions will diffuse through the thin film and gradually eliminate the objectionable ions in the mother liquor. The washing process is allowed to proceed for 10 minutes. The film under the drop is punctured with a carefully sharpened needle and the residue liquor in the drop drains away. Blank tests indicate that the washing and subsequent drainage of the liquid by puncturing are sufficient to remove extraneous ions which would form crystals on the evaporation of the mother liquor. A metal screen is placed over the area of interest and the film plus screen removed to dry in a vacuum at room temperature.

Several modifications of the above technique for preparing crystals have been tried. The first modification is to add one reagent in the form of a crystal to the other reagent as a drop of solution resting on the plastic film over clean distilled water. After precipitation, the crystals are washed and the liquid drained away as described above. A second modification is to add a drop of test solution directly to a drop of reagent placed on the surface of the Parlodion film supported on clean distilled water. This is called the method of direct mixing to distinguish

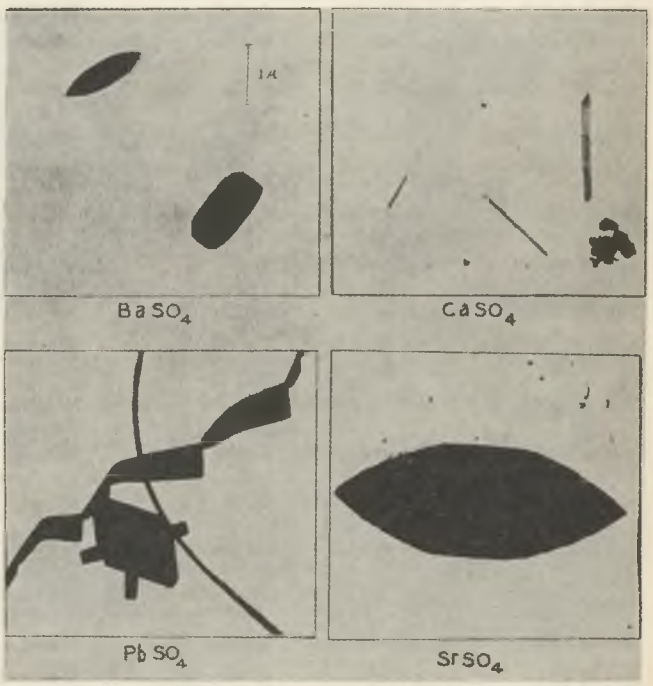

Figure 4. Sulfates of Barium, Calcium, Lead, and Strontium

Reagent, 0.1 *M* K_2SO_4

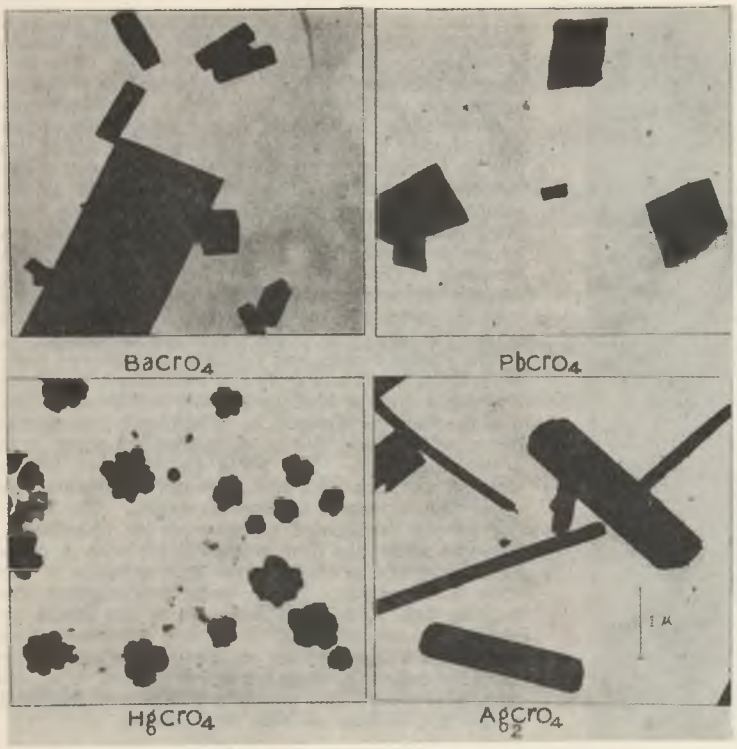

Figure 5. Chromates of Barium, Lead, Mercury, and Silver

Reagent, 0.1 *M* K_2CrO_4

it from precipitation and crystal formation by diffu sion. The direct mixing methods are used for pre paring the more soluble precipitates where consider able material must be diffused through the membrane The diffusion method would require a long time fo diffusion of the required amounts of ions for precipita tion. A slight variation on these latter methods i to precipitate by mixing the two solutions on a spo plate or in a small capillary and then transfer th precipitate plus liquor to a Parlodion film on th water for washing. Most of the studies describe here are made by the diffusion method because o the better control over the precipitation process.

RESULTS

One of the important factors determining th habit of the crystal is the concentration of the test solution. A study of this factor made for the case o silver chromate is shown in Figure 2. The test re agent is 0.1 *M* potassium chromate and 0.001 *M* 0.01 *M*, 0.1 *M*, and 1.0 *M* test solutions of silver nitrate

Table I. Crystals Studied

Test Solution	Reagent	Precipitate	Method	Description of Crystals: Electron microscope	Light microscope	Macroscopic
0.1 *M* $BaCl_2$	0.1 *M* K_2SO_4	$BaSO_4$	Diffusion	Prismatic, tabular with pointed and square ends	Well-defined, tabular (*3*)	Prismatic (*5*)
$Ca(NO_3)_2$ crystal	0.1 *M* K_2SO_4	$CaSO_4.2H_2O$	Mixing	Acicular	Long, slender needles (*3*)	Prismatic to acicular (*5*)
0.01 *M* $Pb(NO_3)_2$	0.1 *M* K_2SO_4	$PbSO_4$	Diffusion	Prismatic, stalactitic	Granular, indistinct (*3*)	Stalactitic, tabular, conic (*5*)
0.1 *M* $Sr(NO_3)_2$	0.1 *M* K_2SO_4	$SrSO_4$	Diffusion	Prismatic, resembles $BaSO_4$	Granular, indistinct (*3*)	Prismatic (*5*)
0.1 *M* $BaCl_2$	0.1 *M* K_2CrO_4	$BaCrO_4$	Diffusion	Prismatic, large and small crystals	Minute grains; square or rectangular plates and tablets (*3*)	.....
0.01 *M* $Pb(NO_3)_2$	0.1 *M* K_2CrO_4	$PbCrO_4$	Diffusion	Prismatic, tabular	Pulverulent (*3*)	Prismatic (*5*)
0.01 *M* $Hg(NO_3)_2$	0.1 *M* K_2CrO_4	$HgCrO_4$	Diffusion	Clusters of poorly developed crystals	No crystalline precipitate (*3*)	.....
0.1 *M* $AgNO_3$	0.1 *M* K_2CrO_4	Ag_2CrO_4	Diffusion	Prismatic, columnar and acicular	Plates, rectangular, elongated (*3*)	.....
0.1 *M* $BaCl_2$	0.1 *M* K_2CO_3	$BaCO_3$	Diffusion	Columnar, pointed, with poorly developed forms	Minute, needles, spider-like aggregates and tiny spherulites (*3*)	Columnar, crystals always repeated twins (*5*)
0.01 *M* $Ca(NO_3)_2$	0.1 *M* K_2CO_3	$CaCO_3$	Diffusion	Outlines suggest rhombohedrons; large crystals and small fibrous ones	Tiny disks and well-formed rhombohedra (*3*)	Variety of habits (*5*)
0.01 *M* $Pb(NO_3)_2$	0.1 *M* K_2CO_3	$PbCO_3$	Diffusion	Hexagonal and irregular outlines	Prismatic (*7*)	Tabular (*5*)
0.1 *M* $Sr(NO_3)_2$	0.1 *M* K_2CO_3	$SrCO_3$	Diffusion	Spear-shaped; poorly defined forms	Spherulites and dumbbell aggregates of tiny needles (*3*)	Spear-shaped, acicular (*5*)
0.1 *M* $CuSO_4$	0.1 *M* K_2CO_3	$Cu(OH)_2.CuCO_3$[a]	Diffusion	Triangular, lamellar prisms grouped as rosettes	.....	Slender, acicular prisms grouped as rosettes (*5*)
0.1 *M* $MnSO_4$	0.1 *M* K_2CO_3	$MnCO_3$	Diffusion	Rounded, irregular, globular	.....	No distinct crystals (*5*)
0.1 *M* $AgNO_3$	0.1 *M* K_2CO_3	Ag_2CO_3	Diffusion	Hexagonal outlines	.....	
0.01 *M* $ZnCl_2$	0.1 *M* K_2CO_3	$ZnCO_3$	Diffusion	Clusters of irregular and fibrous crystals	.....	Rarely well crystallized (*5*)
0.01 *M* $Pb(NO_3)_2$	0.1 *M* Na_2HAsO_4	$Pb_3(AsO_4)_2$[a]	Diffusion	Acicular	Gelatinous precipitate (*3*)	.....
0.01 *M* $Pb(NO_3)_2$	0.1 *M* $(NH_4)_2HPO_4$	$Pb_3(PO_4)_2$	Diffusion	Regular and irregular hexagonal outlines	Curdy precipitate changing to minute rods and needles (*3*)	.....
0.1 *M* $AgNO_3$	0.1 *M* Na_2HAsO_4	Ag_3AsO_4	Diffusion	Hexagonal outlines	Granular precipitate changing to irregular grains and platelets (*3*)	.....
0.1 *M* $AgNO_3$	0.1 *M* $(NH_4)_2HPO_4$	Ag_3PO	Diffusion	Square and rectangular outlines	Granular; tiny dendritic stars, crosses, and radiates (*3*)	.....
.05 *M* $SnCl$	0.1 *M* $Na_2C_2O_4$	SnC_2O_4	Diffusion	Irregular square and parallelogram outlines	Great variety of prismatic or imperfectly developed crystals (*3*)	.....
0.1 *M* $CdSO_4$	0.1 *M* $CdSO_4$	$CdCO_3$	Diffusion	Prismatic, columnar; poorly developed crystals	.....	
0.1 *M* $NiSO_4$	0.1 *M* $(NH_4)_2HPO_4$	$Ni_3(PO_4)_2$	Diffusion	Prismatic and equant	.....	
0.1 *M* $CuSO_4$	0.1 *M* $(NH_4)_2HPO_4$	$Cu_3(PO_4)_2.Cu(OH)_2$[a]	Diffusion	Compacts of irregular pointed crystals	.....	Small prismatic; united in druses (*5*)
0.1 *M* K_2SO_4	5% $H_2PtCl_6.6H_2O$	K_2PtCl_6	Mixing	Hexagonal outlines	Well-formed octahedra (*3*)	Octahedral and cubic forms; well-formed (*7*)
0.1 *M* K_2SO_4	10% $HClO_4$	$KClO_4$	Diffusion	Prismatic, acicular	Prismatic, skeletal (*3*)	.....
0.1 *M* $NiSO_4$	0.5% $C_4H_8N_2O_2$	$Ni(C_4H_7N_2O_2)_2$	Mixing	Long, slender needles	Acicular (*3*)	.....
0.1 *M* $AgNO_3$	0.1 *M* KCNS	AgCNS	Diffusion	Prismatic, columnar	.....	

[a] Composition uncertain.

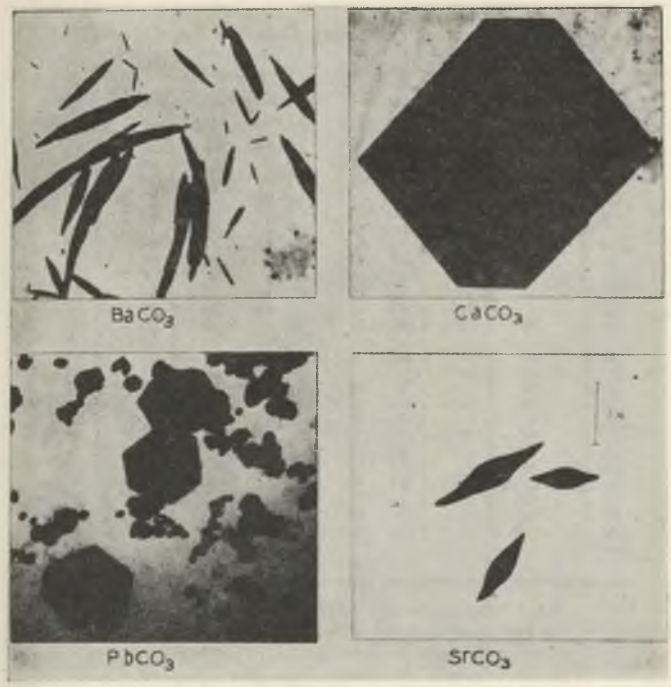

Figure 6. Carbonates of Barium, Calcium, Lead, and Strontium
Reagent, 0.1 *M* K_2CO_3

are used. The formation and growth of the crystal are carried out by the diffusion technique. As the concentration of silver nitrate is increased, both the number and size of the crystal increase. However, for the 1.0 *M* solution, the crystal size decreases.

Although a statistical study was not made on the crystal size as affected by the concentration, observations of different portions of the specimen on the fluorescent screen indicated the tendencies noted. The habit of the crystal is similar for the various concentrations, although the prismatic form of the crystal is enhanced in the case of silver chromate by increasing the concentration. This is seen in the longer crystals which are formed.

Figure 3 shows a comparison of typical crystals obtained by the diffusion technique with those obtained by direct mixing. The effect of concentration of test solution on crystal habit is shown for both cases. For equal concentration of test solution the diffusion method produces the larger crystals; it also produces superior crystals having fewer intergrowths. Increasing the concentration produces smaller crystals by direct mixing while larger crystals are produced by the diffusion method.

A complete study of chemical crystallography would involve: (1) space lattice, (2) habit, (3) number of nuclei, (4) rate of growth, (5) solid solution, and (6) degree of supersaturation. This analysis is difficult and will not be attempted in this paper. However, certain correlations can be made of the chemical composition and the habit of the crystal based on the micro- and macroscopic crystals.

Figure 4 shows the crystal habits obtained for the sulfates of barium, calcium, lead, and strontium, all formed by diffusion precipitation except for calcium sulfate which was formed by the interaction of the test reagent with a crystal. Although the calcium sulfate is probably precipitated as calcium sulfate dihydrate, it is found to be stable to the vacuum and the electron beam in the electron microscope. Table I shows a list of the precipitates obtained in this study; their crystal habits are shown in Figures 4 to 10. The test reagent, test solution, and method of formation are also included.

Figure 5 shows the results obtained with the chromates of barium, lead, mercury, and silver. The carbonates of barium, calcium, lead, and strontium are shown in Figure 6, while Figure 7 shows the carbonates of copper, manganese, silver, and zinc. The arsenates and phosphates of lead and silver are shown in Figure 8, while Figure 9 shows the crystals of stannous oxalate, cadmium carbonate, and nickel and copper phosphate. Figure 10 shows the crystals of potassium perchlorate and chloroplatinate, nickel dimethylglyoxime, and silver thiocyanate.

The crystal habits of the various precipitates are found to be reproducible and, in general, simple habits are obtained. The experimentally observed habits of the crystals are compared in Table I with those given by Chamot and Mason (*3*) and Winchell (*7*) for the light microscope and those given by Dana (*4*, *5*) for macroscopic crystals. Fair agreement is obtained. For gelatinous and granular precipitates the advantage of the high resolving power of the electron microscope is evident. Strontium sulfate, for example, is granular and indistinct in the light microscope but is shown in Figure 4 to have a distinct prismatic habit. The electron micrographs or pictures were taken at 6,700 diameters and then enlarged optically to 16,750 diameters. The size of the crystals can be estimated by comparing the dimensions with the value of 1 micron drawn on each series of prints.

In addition to the experiments described above, the insoluble iodides and oxalates were studied. The iodides were found to melt or decompose in the electron beam. This process could be followed visually on the fluorescent screen. The oxalates decomposed in the microscope or else the crystal forms were not distinctive.

CALCULATIONS

Three calculations will be made: (1) the amount of silver ions precipitated in the experimen tshown in Figure 3, *C*, (2) the distribution of crystals in the specimen and in the photograph, and (3) the theoretical limit to the precipitation of silver ion by the chromate ion.

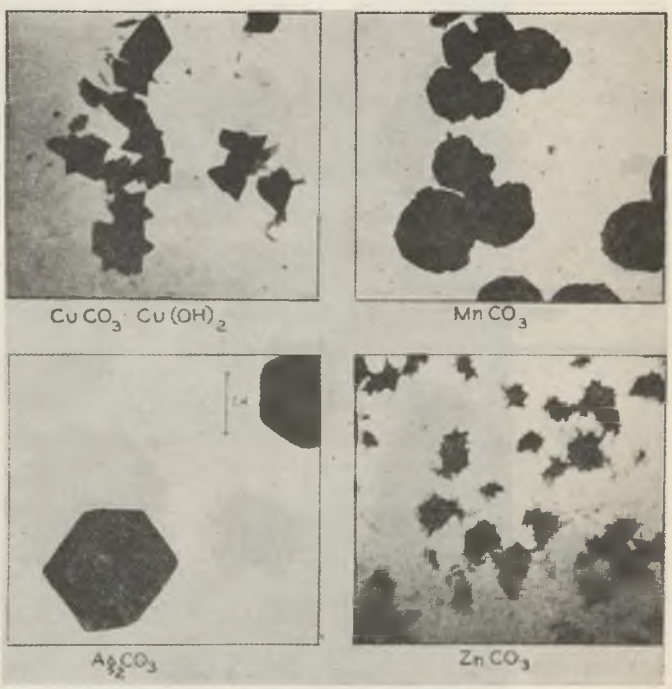

Figure 7. Carbonates of Copper, Manganese, Silver, and Zinc
Reagent, 0.1 *M* K_2CO_3

Table II. Gold Calibration

D, Mm.	I/I_0	d_{hkl}	$D \times d_{hkl}$
11.7	S	2.35	27.5
13.5	M	2.03	27.4
19.1	M	1.439	27.5
22.3	M	1.227	27.4
23.3	W	1.173	27.4
29.3	M	0.935	27.4
30.1	M	0.910	27.4
32.9	W	0.832	27.4
34.9	W	0.784	27.4
		Av.	27.4

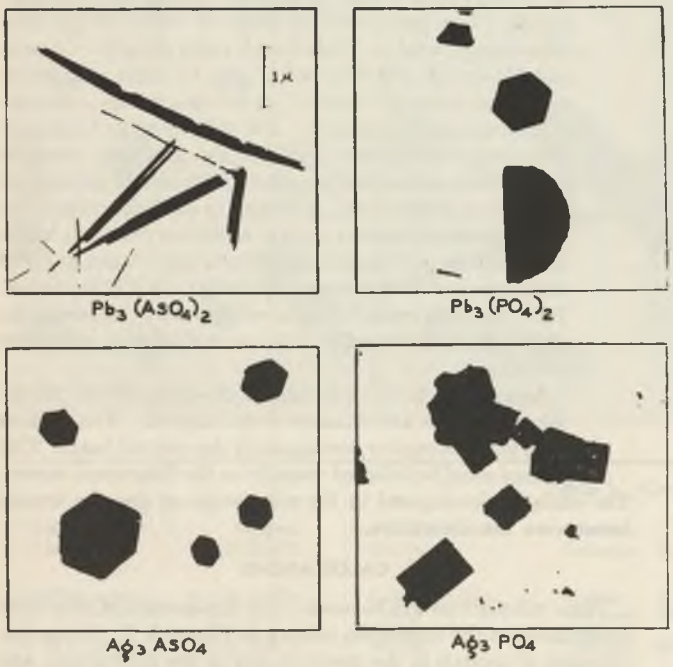

Figure 8. Arsenates and Phosphates of Lead and Silver

Reagents, 0.1 *M* $(NH_4)_2HPO_4$ and 0.1 *M* Na_2HAsO_4

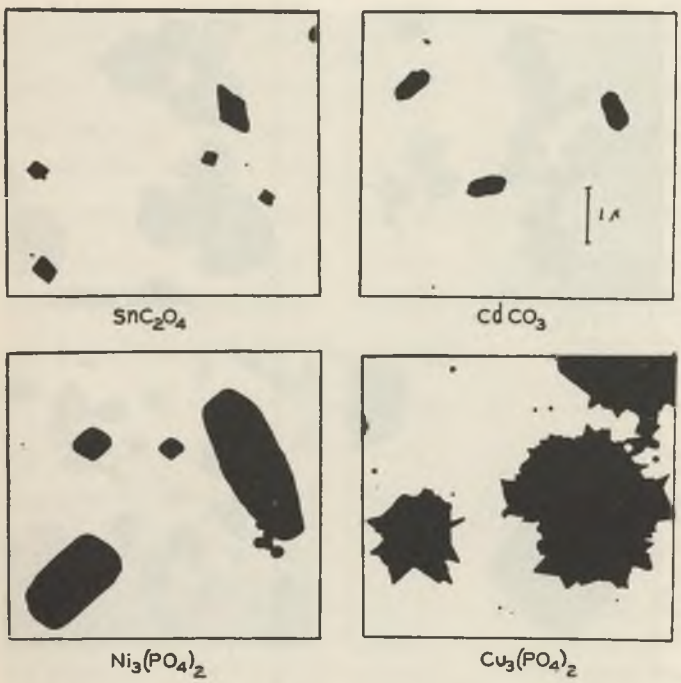

Figure 9. Stannous Oxalate, Cadmium Carbonate, and Nickelous and Cupric Phosphates

Reagents, 0.1 *M* $Na_2C_2O_4$, 0.1 *M* K_2CO_3, and 0.1 *M* $(NH_4)_2HPO_4$

Table III. Electron Diffraction Data on $BaCrO_4$ and $BaSO_4$

$BaCrO_4$					$BaSO_4$				
Experimental			X-Ray tables		Experimental			X-Ray tables	
D, mm.	I/I_0	d_{hkl}	d_{hkl}	I/I_0	D, mm.	I/I_0	d_{hkl}	d_{hkl}	I/I_0
6.9	M	3.97	4.00	M	6.35	W	4.30	4.35	M
7.5	M	3.65	..	..	7.0	M	3.90	3.89	M
7.8	S	3.52	3.54	S	7.65	W	3.57	3.57	W
8.1	M	3.38	..	..	7.95	S	3.43	3.44	S
8.7	S	3.15	3.19	S	8.3	W	3.29	3.31	M
9.5	S	2.89	2.90	S	8.8	S	3.10	3.10	S
9.9	W	2.77	2.78	M	9.65	M	2.83	2.83	M
10.7	W	2.56	2.53	W	10.1	M	2.71	2.72	M
11.6	W	2.37	2.37	W	10.6	M	2.57	..	..
12.2	W	2.25	2.25	W	11.15	W	2.45	2.47	W
12.7	S	2.16	2.16	S	11.85	W	2.30	2.31	W
14.5	W	1.89	1.91	M	12.4	M	2.20	2.20	W
					13.0	S	2.10	2.10	S
14.9	W	1.84	1.80	W	13.3	W	2.05	2.04	W
15.2	W	1.80	..	..	14.2	W	1.92	1.92	W
16.0	M	1.71	1.71	M	14.9	W	1.83	1.85	W
16.5	W	1.66	1.66	W	15.9	W	1.72	1.74	W
16.9	W	1.62	1.62	W	16.85	W	1.62	1.63	W
17.5	W	1.57	1.56	W	17.2	W	1.59	1.58	W
18.9	W	1.45	1.45	W	17.85	W	1.53	1.52	M
19.4	W	1.41	1.41	W	19.2	W	1.42	1.42	W
21.2	W	1.29	1.29	W	19.4	W	1.41	..	..
22.2	W	1.24	1.25	W	21.6	W	1.26	1.26	W
23.4	W	1.17	1.17	W	24.9	W	1.10	1.09	W
24.4	W	1.12	1.12	W	27.7	W	0.987	..	..
					30.3	W	0.902	..	..

1. Silver nitrate, 0.001 *M*, is reacted with 0.1 *M* potassium chromate by diffusion. Sample volume is approximately 10^{-3} cc. or 1 cu. mm. This volume contains 1.08×10^{-7} gram of silver ions per cu. mm. This silver produces crystals upon diffusion of chromate into the sample volume—some are shown in Figure 3, *C*. The method in this case has detected 1.08×10^{-7} gram of silver.

2. The specimen area is 1 sq. mm. and 1 cu. mm. is used for the sample. Assume uniform distribution of crystals over the specimen area and also that the resulting silver chromate crystal is about 1 micron long, 0.5 micron wide and deep. This follows from analysis of crystal shown in Figure 3, *C*, and from the assumption that the crystal is as deep as it is wide. Weight of crystal is 1.41×10^{-12} gram and it contains about 0.91×10^{-12} gram of silver. If the silver ions are precipitated as silver chromate crystals of a uniform size, a total of about 118,000 crystals would be formed over the 1 sq. mm. area.

The area of specimen from which the micrograph was photographed (6700×) represents about 1/18,000 sq. mm. Therefore the average number of crystals found in the electron microscope on any 4 square inch area (2 × 2) is 6.5. This is ample. Specimens having one tenth or one hundredth of this number may be used, since the specimen can be moved on the stage in the electron microscope.

3. The theoretical limit to the detection of silver ion by the chromate ion can be estimated. The formation of crystals by the diffusion method is limited by the solubility product and by the time for the crystal to grow. The diffusion of the potassium and chromate ions into the drop is nearly independent of the silver and nitrate ion concentrations at low silver nitrate concentrations. However, the crystal nuclei must be formed and grow before the silver nitrate diffuses out of the drop and into the chromate solution. The solubility product of silver chromate is 9×10^{-12} at 25° C. If a 1 *M* potassium chromate solution is used as a reagent, the limiting concentration on the silver ion is 3×10^{-6} mole per liter or 3.24×10^{-10} gram per cu. mm. Therefore the method may detect one tenth or one hundredth the amount of silver specified in part 1 or 10^{-8} or 10^{-9} gram of silver. Below these figures solubility product considerations enter and difficulties may be encountered in finding crystals for study.

Similar calculations can be made for the other systems studied.

ELECTRON DIFFRACTION

An additional feature of the electron microscope, which is of considerable value in identifying the nature of the crystal, is the use of the electron diffraction adapter. For this purpose a heavier concentration of crystals is necessary, and the growth of well-formed crystals is not important. The technique of using the electron diffraction adapter has been described by Picard (*6*).

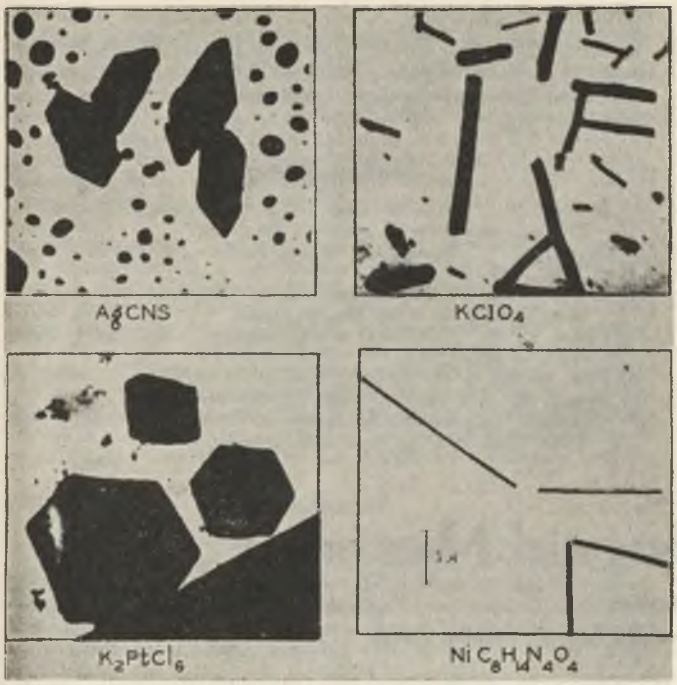

Figure 10. Silver Thiocyanate, Potassium Perchlorate and Chloroplatinate, and Nickel Dimethylglyoxime

Figure 11 shows typical diffraction pictures of barium chromate, barium sulfate, gold, and a Parlodion film. The barium chromate and barium sulfate are prepared by the diffusion technique, while the gold is formed by evaporation on to a Parlodion film. The gold picture is included for calibration purposes and is taken at the same time as the other pictures in the group. This calibration procedure is necessary because of the change in the absolute value of the accelerating voltage and also the change of the current in the diffraction focusing lens.

Table II shows the data obtained from the gold calibration. D refers to the diameter of the particular diffraction ring. The ratio I/I_0 is an estimate of the relative intensities of the several lines. S refers to strong, M to medium, and W to weak. The lattice spacings d_{hkl} are taken from x-ray diffraction tables. The calibration constant ($D \times d_{hkl}$) is found to be 27.4.

Table III shows the electron diffraction data obtained from barium chromate and barium sulfate crystals. The data obtained from x-ray tables are included for comparison; the agreement is good. The value of these data is apparent from the table.

CONCLUSIONS

A technique for the controlled growth of crystals on thin plastic films is based on the diffusion of ions and molecules through the plastic film and subsequent precipitation on the film.

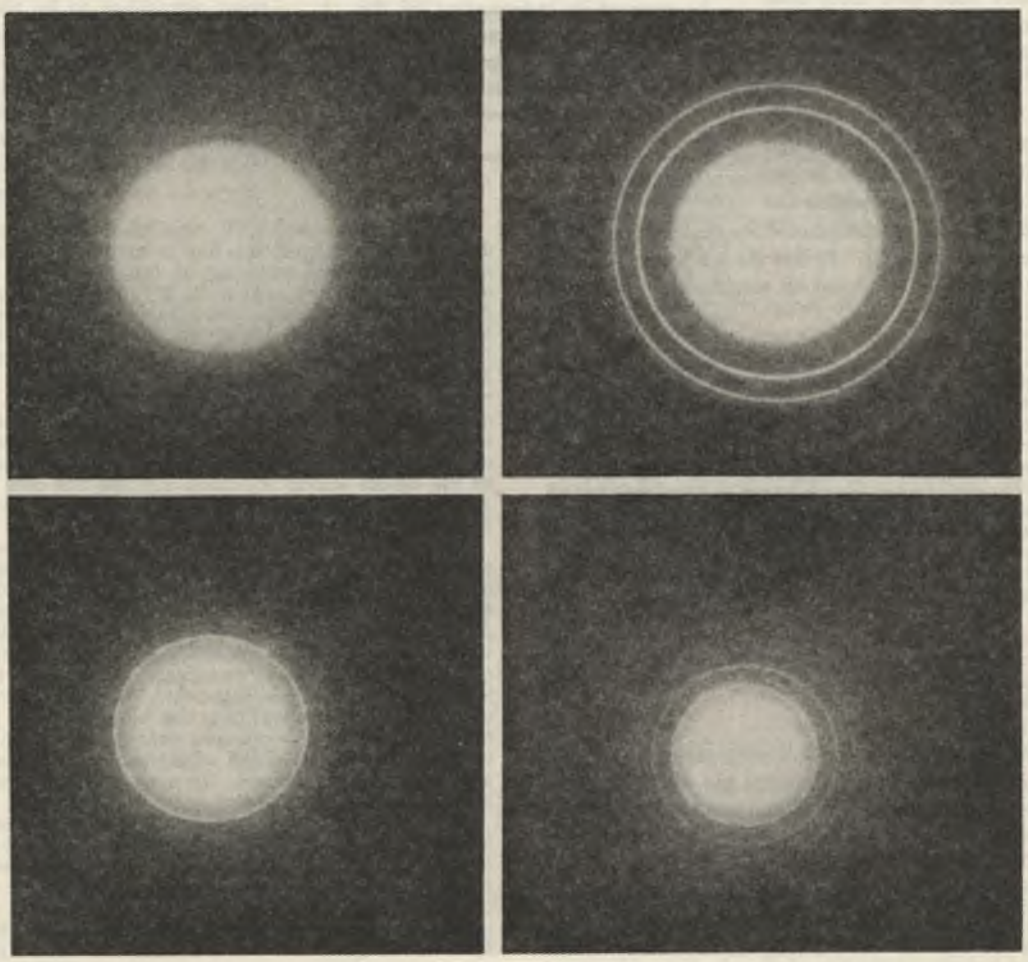

Figure 11. Diffraction Patterns of Parlodion, Evaporated Gold, Barium Sulfate, and Barium Chromate

The diffusion method offers better control of the crystal size and shape than preparing crystals by direct mixing.

Although the crystal size depends upon the concentration, the habit is only slightly affected.

A considerable number of crystals were prepared and electron micrographs taken. When the habit of the crystals was compared with that observed for micro- and macroscopic crystals, good agreement was observed.

Calculations for a typical reaction indicate that quantities of the order of 10^{-7} gram of silver ion can be detected in reaction with a 0.1 M potassium chromate solution. The theoretical limitation is approximately 10^{-8} or 10^{-9} gram of silver ion in the same reaction.

Electron diffraction analysis is utilized to help in identification of the crystal.

The application of the electron microscope to chemical microscopy is suggested. A useful role may be expected, especially in the study of precipitates which are not perceptible under the optical microscope. The practical details of individual reactions have yet to be developed. They would include a study of concentration limits, interferences, trials in the identification of real unknowns, etc.

LITERATURE CITED

(1) Boricky, E., "Elemente einer neuen chemischmikroskopischen Mineral- und Gestein-Analyse", Prag, 1877.
(2) Burton, C. J., Barnes, R. B., and Rochow, T. G., IND. ENG. CHEM., 34, 1429 (1942).
(3) Chamot and Mason, "Handbook of Chemical Microscopy", New York, John Wiley & Sons, 1940.
(4) Dana, E. S., "Textbook of Mineralogy", New York, John Wiley & Sons, 1915.
(5) *Ibid.*, revised by W. E. Ford, 1940.
(6) Picard, R. G., *J. Applied Phys.*, 15, 678–84 (1944).
(7) Winchell, "Microscopic Characters of Artificial Minerals", New York, John Wiley & Sons, 1931.

An Automatic Differential Manometer

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The author describes a gage for measuring pressure changes with an accuracy of 0.01 mm. when the total pressure is 50 mm. or more. The sensitivity of the instrument is independent of the total pressure and mercury is used as the confining liquid. Pressure changes are read directly off a scale graduated in millimeters.

THE problem of measuring small pressure changes in gaseous reactions has always presented considerable difficulty to the physical chemist. The McLeod gage has probably been used more than any other instrument for this purpose but it can be used only at relatively small total pressures. Bourdon and spoon gages have been used in many cases but they are of necessity very fragile if pressure changes of the order of 0.01 mm. are to be detected. In the opinion of the author the most satisfactory differential manometer for general laboratory use is that due to Pearson (*4*). It has the advantage of using mercury as the confining liquid and its sensitivity is independent of the total pressure.

The principle on which the gage operates is simple. Consider three vertical glass tubes, T_1, T_2, and T_3, joined together at the bottom and containing mercury. If T_1 is evacuated and T_2 is connected to the system whose pressure changes are to be measured, and if h_1, h_2, and h_3 are the corresponding heights of the mercury above a fixed horizontal plane, then the pressure in the system is obviously $h_1 - h_2$. Furthermore if, by some method, we are to maintain level h_1 constant, any change in pressure in the system must be compensated by a pressure change in tube T_3. Since there is a fixed amount of mercury in the gage, $\Delta h_3 / - \Delta h_2$ must be equal to $(d_2/d_3)^2$, where d_2 and d_3 are the internal diameters of tubes T_2 and T_3, respectively; or $\Delta p = (d_3/d_2)^2 \times \Delta h_3$, where p is the gas pressure in T_2. In practice d_2 may be 20 to 25 mm. and d_3 1 to 2 mm. The pressure change in T_2 can thus be magnified several hundred times. The two limiting factors are the method of indicating a constant level in T_1 and the method of controlling the pressure in T_3.

Blacet and his associates (*1*, *2*) have used this type of gage successfully in their work on photochemical gas reactions. The correct height of the mercury in T_1 was indicated by the making and breaking of an electrical contact between the mercury surface and a fine tungsten wire, the circuit being completed through a second tungsten wire sealed through the tube below the mercury surface. The pressure in T_3 was controlled manually by turning a three-way stopcock, either evacuating or admitting air to a large ballast volume at the top of T_3. In the author's experience this method of control was unsatisfactory for three reasons: (1) the turning of the stopcock communicated unde sirable vibrations to the mercury surfaces; (2) it was difficult to adjust the pressure in T_3 to the correct value manually; and (3) it would be a great advantage to be able to read the pressure at any time without having to manipulate the controls.

The gage in its present form is illustrated in Figure 1. It is similar in construction to that of Blacet *et al.* (*1*, *2*) with the exception of the automatic feature and a few minor changes.

The tungsten needle, W, was made from a piece of 1-mm diameter wire shaped to a fine point by treatment with sodium nitrite. Accurate centering of the needle was facilitated by sealing it as shown into a 10/30 ground joint lubricated with Apiezon N grease. The two electrical contacts were connected to a vacuum tube relay of the type described by Serfass (*5*). No time delay was used. The relay operated a fixed solenoid, S. When the solenoid is actuated the glass-enclosed iron plunger is raised and air enters the 500-cc. bulb, B, through the thin Alundum disk, D, sealed to the bottom of the 6-mm. diameter Pyrex tube. When the relay current is cut off the plunger falls, shutting off the air supply. The success of the instrument is largely due to the needle valve, N, which controls the rate of evacuation. After some investigation Hoke high-vacuum valve No. 318 was found to be most satisfactory.

For a porous disk, D, of fixed dimensions and with continuous pumping through N (a Hyvac pump was used) there is an equilibrium pressure for B where the rate at which air enters through D is equal to the rate at which it is pumped out through N. If this equilibrium pressure happens to be in the range where the gage is supposed to operate, control will be impossible. Consequently, it is necessary to adjust N so that the rate at which air is pumped away will be somewhat less than the rate at which it can enter (with D open) over the whole range of pressures to be encountered. If the pressure change during an experiment is large, an occasional slight adjustment of the needle valve will assure control at all times. In most of the author's experiments no readjustment was necessary.

In one model T_2 was approximately 22 mm. and T_3 2.0 mm. in inside diameter. T_2 was calibrated over a range of 100 mm. before sealing it into the apparatus. Since the pressure change in the system during a run was only of the order of 1 mm., the value of d_2 would remain practically constant. T_3 was calibrated over a range of about 300 mm., corresponding to a pressure in B of from 250 to 550 mm. For a nonuniform bore the expression for the pressure change becomes $\Delta p = \frac{\int d_3^2 \times dh_3}{d_2^2}$ where the

integral is taken over the range of variation of h_3, from the value at the beginning of the run to the value at the time of observation. Although the internal diameter of T_1 does not appear in the expression for Δp, its value does affect the behavior of the instrument. Since the mercury level in T_1 continually fluctuates as contact with the needle is made and broken, it is desirable to have d_1 small to avoid large fluctuations in h_3; however, if it is too small the mercury will have a tendency to stick, and if it is too large, vibrations on the mercury surface will be serious. In the instrument described here d_1 was approximately 18 mm. in inside diameter.

The only other dimension that need be mentioned is the distance from the tip of the needle, W, to the top of the mercury surface in R. This was approximately 800 mm., so that only a slight pressure had to be applied to bring the mercury in contact with the needle before closing P, as described below. Valve N and the U-tube and solenoid can be placed in any convenient position. The construction of the top of bulb B is such as to prevent mercury from capillary T_3 from getting on top of the Alundum disk, D, or into valve N through an accidental large increase in pressure in the system. When not in use the manometer was isolated from the rest of the system by a mercury cutoff.

PROCEDURE

By connecting reservoir R to a vacuum line by means of the three-way stopcock the mercury level in T_1 and T_2 is brought down to a point somewhat below the junction of these two tubes. Stopcock P is open. T_1 and T_2 are then evacuated and flamed, if necessary, together with the remainder of the system connected to the top of T_2. When a stable vacuum of 10^{-5} to 10^{-6} mm. is attained, the mercury is admitted to T_1 and T_2 by connecting the three-way stopcock to the atmosphere. The gas is then introduced into the reaction system at the desired pressure, forcing the mercury down in T_2 and up in T_1. The pressure in B is adjusted until the mercury level in T_3 is in a suitable part of the tube. By means of an aspirator bulb connected to the three-way stopcock on R the mercury level in T_1 is raised until it coincides approximately with the tip of the needle. Stopcock P is now closed and the relay circuit switched on. When the needle valve is properly adjusted the gage is completely automatic. The mercury in T_3 will rise or fall until contact is made in T_1. Small vibrations on the mercury surface cause the relay to go on and off a hundred or more times a minute, but the level in T_3 remains sensibly constant to within a fraction of a millimeter. The position of the mercury in T_3 is noted and the run can then be started. Subsequent changes in h_3 are related to pressure changes in the reaction system by the relation $\Delta p = \frac{d_3^2}{d_2} \times \Delta h_3$ or, if T_3 is not uniform, $\Delta p = \frac{\int d_3^2 \times dh_3}{d_2^2}$.

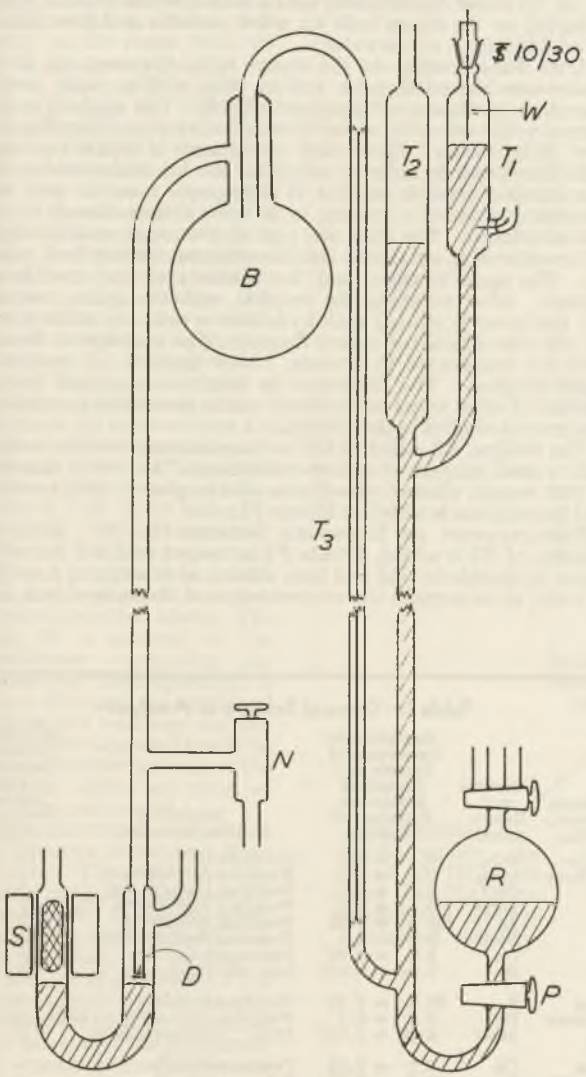

Figure 1. Gage

The use of this type of gage by the author has already been referred to (*3*). Its use in other researches as well has shown it to be almost indispensable in the investigation of gaseous reactions involving small pressure changes. The maximum value of $(d_2/d_3)^2$ used so far is about 100, although larger ratios could be used (*1*, *2*).

Temperature changes must, of course, be taken into consideration if the measurements are to have any meaning. The author has done this in two ways: (1) by immersing the system, not including the manometer, in a thermostatically controlled bath, and (2) by observing the temperature of the system at the time of each pressure reading and making the necessary corrections. In addition to actual pressure changes in the system due to its temperature, the manometer itself is subject to an error resulting from the change in volume of the mercury. The magnitude of this latter error can be calculated as follows:

If V_0 is the volume of mercury in the gage (not including that in reservoir R) when the pressure in the system is zero and if V is the corresponding volume when the pressure is p cm., then

$$V = V_0 - pA_2$$

where A_2 is the cross-sectional area of T_2. The increase in h_3 for a temperature increase Δt is the sum of two quantities resulting from the increase in volume of the mercury and the decrease in the density of the mercury in a column of length p. The first quantity is $\frac{1}{A_3} \frac{dV}{dt} \times \Delta t$ and the second is $\frac{A_2}{A_3} \times \frac{p}{V} \frac{dV}{dt} \times \Delta t$

For mercury $\frac{dV}{dt} = 1.8 \times 10^{-4} V$

Therefore $\frac{dh_3}{dt} = \frac{1.8 \times 10^{-4}}{A_3}(V + pA_2) = \frac{1.8 \times 10^{-4} V_0}{A_3}$

In the manometer described here V_0 was approximately 115 cc. and A_3 0.03 sq. cm., the error in h_3 due to a change in temperature of the mercury is about 6.6 mm. per degree, corresponding to a pressure error of approximately 0.08 mm. per degree. It follows that the temperature of the manometer must not be permitted to change more than a few tenths of a degree during the course of a run and that the volume of mercury must be kept at a minimum. The latter is accomplished by using small-diameter tubing in all places except in the part of T_1 adjacent to the tip of the needle and in the part of T_2 covering the range of pressures to be encountered.

LITERATURE CITED

(1) Blacet, F. E., and Volman, D. H., *J. Am. Chem. Soc.*, **61**, 582 (1939).
(2) Leighton, P. A., and Blacet, F. E., *Ibid.*, **54**, 3165 (1932).
(3) LeRoy, D. J., and Steacie, E. W. R., *J. Chem. Phys.*, **12**, 117 (1944).
(4) Pearson, T. G., *Z. physik. Chem.*, **A156**, 86 (1931).
(5) Serfass, E. J., IND. ENG. CHEM., ANAL. ED., **13**, 262 (1941).

CORRECTION. The article "Microdetermination of Carbon and Hydrogen" by Ralph O. Clark and Gordon H. Stillson [IND. ENG. CHEM., ANAL. ED., **17**, 520 (1945)] was presented at the 107th Meeting of the AMERICAN CHEMICAL SOCIETY, Cleveland, Ohio, and not at the New York Meeting, as stated in the footnote.

Microchemical Analysis of Sphalerite from Kristineberg, Sweden

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Because the presence of iron as marmatite in sphalerite may cause serious loss of zinc, knowledge of the amount of iron remaining after flotation is important. Difficulty in collecting samples of pure sphalerite made desirable application of micromethods to the analysis of six samples of sphalerite from the Kristineberg mine in Sweden, varying in color from pale yellow to brown, the procedure used is presented here in detail. The iron content was found to vary from 1 to 8.5%, corresponding to a variation of 65 to 58% in zinc content. The normal composition of sphalerite from the Kristineberg mine is approximately 64% zinc, 2.5% iron, 0.2% cadmium, and 33% sulfur.

THE analytical work reported in this paper was part of an unpublished investigation at the Kristineberg mine made by T. Du Rietz. Knowledge of the chemical composition of the purest sphalerite which might be separated from the ore by mechanical means (flotation) is of obvious practical interest. Great difficulty was experienced, however, in collecting samples of reasonably pure sphalerite from the ore, and it became advisable to apply micromethods of chemical analysis to 0.08- to 0.03-gram samples.

The sphalerite in the ore of the Kristineberg mine shows gradation of color from a bright yellow transparent type to an opaque brown type. Pyrrhotite, Fe_nS_{n+1}, is very rare in the Kristineberg ore, but the sphalerite may contain minute inclusions of pyrite, FeS_2, and may hold relatively large amounts of iron in the form of marmatite, a solid solution of ferrous sulfide in zinc sulfide. Iron present as marmatite has a pronounced tendency to form zinc ferrite, $ZnO.Fe_2O_3$ during the roasting of the sphalerite and to cause serious losses of zinc due to the insolubility of the ferrite in the subsequent extraction of the roasted ore with sulfuric acid. Consequently, knowledge of the amount of iron remaining in the sphalerite after separation by flotation held sufficient interest to suggest analysis of various color types of the sphalerite.

SCHEME OF ANALYSIS AND SIZE OF SAMPLES

Qualitative spectrographic analysis of six samples of sphalerite by Mr. Alvfeldt of this laboratory indicated the desirability of determining the quantities of silicon, aluminum, manganese, calcium, magnesium, copper, cadmium, bismuth, lead, and phosphorus in addition to zinc, sulfur, and iron. Silver and arsenic were found in such small quantities as to eliminate the necessity of their quantitative estimation; this left thirteen elements needing quantitative determination.

The necessity of conserving material suggested a scheme of analysis permitting the determination of a large number of elements in each portion of the sample. The minimum size of each portion of sample was calculated as a function of precision requirements and the limitations were set by sample, method, and measuring devices (*2*). The average deviation of a single weighing with the available Kuhlmann balance was found equal to ±0.003 mg., and it was reasoned that all the determinations required could be carried out on three portions of the sample as outlined in Table I. A total of 17 + 5 + 5 = 27 mg. of sphalerite should suffice for complete analysis, and 0.06 gram of sample should allow two determinations of each element.

DETERMINATION OF SILICA, COPPER, BISMUTH, ZINC, IRON, ALUMINA, CALCIUM OXIDE, AND MAGNESIA

A brief outline of the scheme of analysis is given in Table II. The procedure is described below, followed by a discussion of the reasons for certain of its features.

SOLUTION OF SAMPLE AND DETERMINATION OF SILICA. Approximately 15 mg. of the sample are accurately weighed in a 5-ml. microbeaker of chemically resistant glass (*3*). The beaker is covered with a watch glass or glass bulb, and its contents are treated with 0.5 ml. of 12 molar hydrochloric acid (sp. gr. 1.19) and heated on the steam bath. When the reaction ceases, 0.01 ml. of 14.5 molar nitric acid (sp. gr. 1.40) is added, and the heating is continued for a few more minutes. Then the cover is rinsed and removed, and the contents of the beaker are evaporated to dryness. The residue is treated with 0.2 ml. of 7.3 molar hydrochloric acid (sp. gr. 1.12). Evaporation to dryness is repeated, whereupon the beaker and contents are heated 30 minutes at 105° C. for dehydration of the silica. After cooling to room temperature, 0.1 ml. of 7.3 molar hydrochloric acid is added to the residue, which is heated on the steam bath for a few minutes and then diluted by adding 0.5 ml. of hot water.

After brief heating on the steam bath, the contents of the beaker are filtered through a filter stick with a paper mat as described by Schwarz-Bergkampf (*3*, *22*). The washing is performed with 1 ml. of 0.1 molar hydrochloric acid and, finally, with 2 ml. of hot water. Filter stick and interior of beaker are wiped with filter paper in order to collect all the insoluble residue, *R*1. The smallest possible amount of filter paper must be used, and its total area must be known, so as to be able to correct for the ash introduced. The filter mat and all the paper used in wiping are transferred to a platinum crucible of approximately 3-ml. capacity. The paper is ashed, and the contents of the crucible are ignited. After weighing the crucible with the ignited residue, *R*1, the latter is treated with hydrofluoric acid and sulfuric acid for the volatilization of silicon fluoride. The contents of the crucible are evaporated to dryness. After ignition the crucible is again weighed. The difference in weight is corrected for the amount of silica found in the blank, and is then taken to represent the amount of silica in the material.

The residue, *R*2, which is left in the platinum crucible, is fused with a small amount of sodium carbonate. The melt is dissolved in just enough dilute hydrochloric acid to give an acid reaction, and the solution is added to filtrate *F*1.

PRECIPITATION OF HYDROGEN SULFIDE GROUP. After the solution of *R*2 is added, filtrate *F*1 is treated with 0.3 ml. of 7.3 molar hydrochloric acid and then diluted with water to a volume of 5 ml., so as to make the concentration of the hydrochloric acid

Table I. General Scheme of Analysis

Sample Taken, Mg.	Constituent Determined	Approximate Percentage in Sample and Permitted Maximum Variation of Findings	Method of Determination	Minimum Sample Required to Give Desired Precision, Mg.
17 or more	SiO_2	0.5 ± 0.1	Weighing SiO_2	17
	Al_2O_3	0.5 ± 0.1	Weighing $Al(C_9H_6NO)_3$	2
	CaO	0.5 ± 0.1	Weighing $CaC_2O_4.H_2O$	6
	MgO	0.5 ± 0.1	Weighing $MgNH_4PO_4.6H_2O$	3
	Zn	50.0 ± 0.25	Weighing $ZnNH_4PO_4$	5
	Fe	2.0 ± 0.1	Weighing $Fe(C_9H_6NO)_3$	2
	Cu	0.2 ± 0.02	Iodometric titration	5
	Bi	0.05 ± 0.005	BiI_3, colorimetrically	16
5 or more	S	30.0 ± 0.25	Weighing $BaSO_4$	2
	P_2O_5	0.5 ± 0.1	Weighing $(NH_4)_3PO_4.12MoO_3$	0.7
	MnO	0.02 ± 0.002	MnO_4^-, colorimetrically	5
5 or more	Cd	0.2 ± 0.02	Polarographically	5
	Pb	0.2 ± 0.02	Polarographically	5

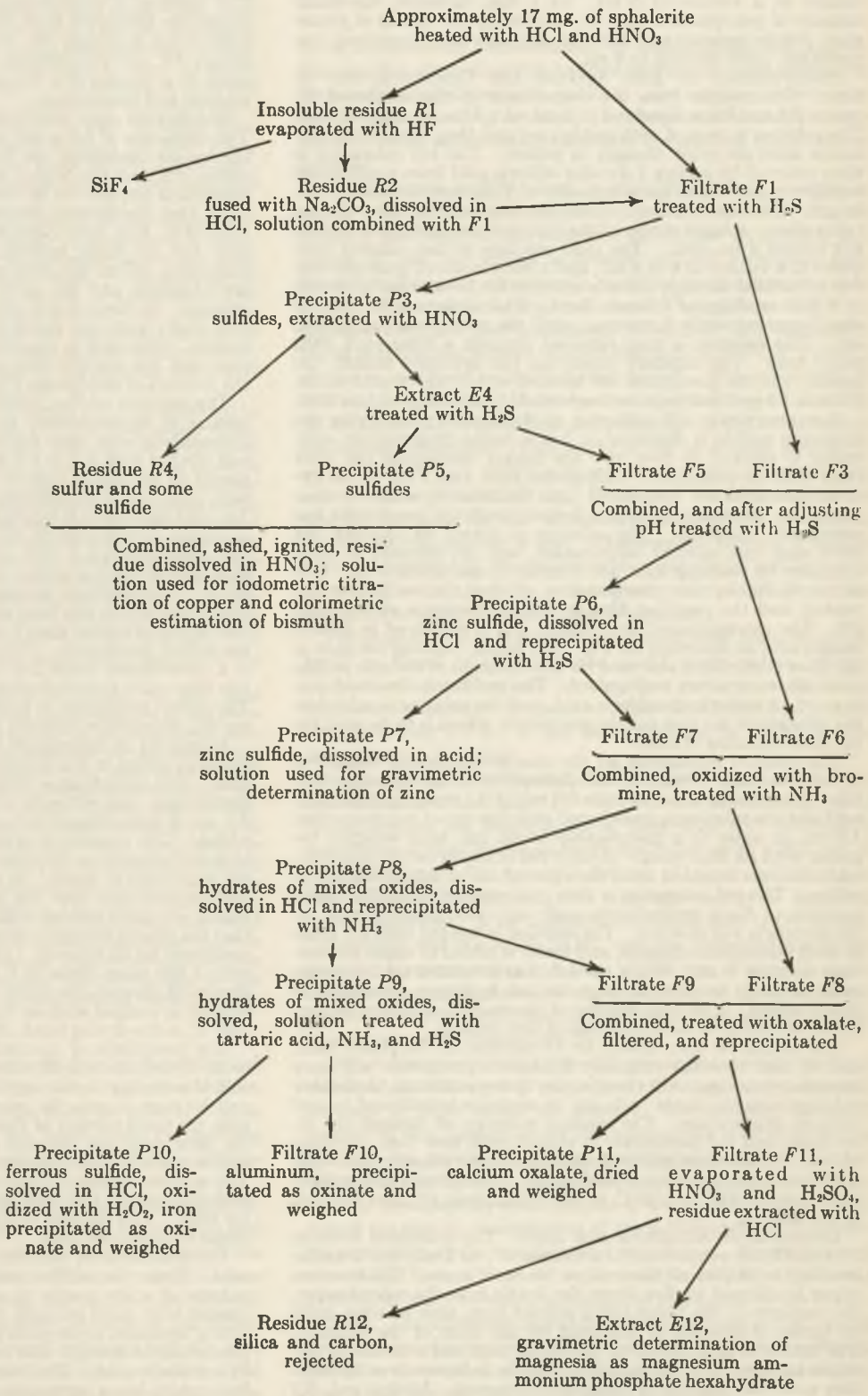
in the solution equal to 0.6 molar. The solution is then heated on the steam bath. Hydrogen sulfide is bubbled through the hot solution by means of a fine capillary of 0.1-mm. bore (*3*), and the solution is saturated with the gas while it is allowed to cool to room temperature. The precipitation requires 5 to 10 minutes. Filtrate *F3* is sucked off through a glass filter stick provided with a mat of filter paper (*3*, *22*). The capillary supplying the hydrogen sulfide and the microbeaker are washed with dilute (0.3 molar) hydrochloric acid which has been saturated with hydrogen sulfide. Filtrate *F3* and washings are collected in a microbeaker of 10-ml. capacity and immediately transferred to a steam bath and evaporated to dryness.

Precipitate *P3*, which may contain some zinc and iron, is dissolved as follows: The capillary supplying the hydrogen sulfide is wiped with a small square of filter paper to collect any precipitate adhering to the paper, which is then placed in the microbeaker containing precipitate *P3* and the filter stick with its paper mat still in place. One milliliter of 3 molar nitric acid (sp. gr. 1.10) is introduced into the microbeaker, which is then heated on the steam bath until all the precipitate is dissolved. The solution, *E4*, is sucked through the filter stick and collected in another microbeaker. Transfer of the extract is made complete by washing with hot water. The paper mat of the filter stick and the piece of filter paper remaining in the microbeaker are transferred to a porcelain crucible together with residue *R4*, mostly sulfur, transfer of which is made complete, if necessary, by wiping the filter stick and interior of the beaker with a small square of filter paper.

Extract *E4* and washings are evaporated to dryness so as to eliminate most of the nitric acid, and the residue is dissolved in 5 ml. of 0.6 molar hydrochloric acid. The solution is treated with hydrogen sulfide, and precipitate and solution are separated and collected as described above. Filtrate *F5* is received in the microbeaker containing the residue from the evaporation of filtrate *F3*, and the combined filtrates and washings are immediately evaporated nearly to dryness in order to remove the hydrogen sulfide and most of the hydrochloric acid.

DETERMINATION OF COPPER AND BISMUTH IN SULFIDE PRECIPITATE. The paper mat containing most of precipitate *P5* is transferred to the porcelain crucible in which residue *R4* has been collected. If necessary, the transfer of precipitate *P5* is made complete by the use of a small square of filter paper. A small amount of the precipitate may remain behind in the microbeaker. The contents of the crucible are ashed and ignited. Any precipitate which may have remained in the microbeaker is dissolved by heating with a small volume of dilute nitric acid. The solution is transferred to the crucible containing the ignited residue. The contents of the crucible are heated on the steam bath and then evaporated to dryness. The residue is dis-

Table II. Scheme for Determination of Silica, Alumina, Iron, Calcium, Magnesium, Copper, Bismuth, and Zinc



solved in 5 cu. mm. of 14.5 molar nitric acid. The solution is diluted with 0.5 ml. of hot water. It is first made slightly ammoniacal, then acidified with acetic acid, treated with potassium iodide and starch solution, and titrated with 0.01 *N* sodium thiosulfate as described in a previous publication for the determination of copper (*3*, *16*).

If a significant amount of bismuth is present, the solution will be distinctly yellow when the end point is reached in the titration of the copper. The solution is used for the colorimetric estimation of bismuth. If there is a precipitate of cuprous iodide, the solution is passed through a filter stick into a beaker and then diluted to a volume of 5 ml. As a rule, however, the small amount of precipitate is held in solution by the excess of potassium iodide present, and the bismuth may be determined immediately after diluting to 5 ml.

Precipitation of Zinc Sulfide and Determination of Zinc. The residue from the evaporation of the combined filtrates, *F*3 and *F*5, is dissolved in 1 ml. of 0.5 molar sulfuric acid. The solution is treated with methyl red and then with dilute ammonia until the color changes to yellow. The red coloration is then restored by adding 1.8 molar sulfuric acid from a calibrated capillary pipet which has a capacity of 5 cu. mm. for each 1-cm. length. The acid is added in small portions until the color change takes place; a small excess of the acid is permissible, but it must not exceed 8 cu. mm. The clear solution is then diluted with water to a volume of 6 to 8 ml., and a fast stream of hydrogen sulfide is bubbled through the cold solution for 10 minutes by means of a fine capillary of 0.1-mm. bore. The mixture is allowed to stand approximately 5 minutes, until the precipitate has settled out. The solution is then removed through a filter stick with paper mat. The capillary supplying the hydrogen sulfide, the beaker, and the precipitate are washed with 1 ml. of cold water. Filtrate *F*6 and washings are evaporated to a volume of 1 to 2 ml.

Any precipitate, *P*6, adhering to the capillary supplying the hydrogen sulfide is dissolved in hot 4 molar hydrochloric acid, which is allowed to flow into the beaker containing the filter stick with the precipitate. Altogether from 1 to 2 ml. of the hydrochloric acid are introduced into the beaker, which is then placed on the steam bath and heated until the precipitate has completely dissolved. The solution is drawn off through the filter stick into another microbeaker, and the transfer is made complete by washing with hot 1 molar hydrochloric acid. If it seems desirable, the precipitation of the zinc sulfide is repeated, and the resulting filtrate, *F*7, is combined with filtrate *F*6. The reprecipitated zinc sulfide, *P*7, is dissolved in hydrochloric acid as described for *P*6. The paper, forming the mat of the filter stick which was used for the filtration of the zinc sulfide precipitates, is ashed and ignited. The residue is dissolved in a drop of 12 molar hydrochloric acid, and the solution is added to the solution of the zinc sulfide precipitate, which is to be used for the determination of zinc.

The solution containing all the zinc is evaporated almost to dryness to make certain of the elimination of the hydrogen sulfide. The residue is dissolved in 0.5 ml. of 0.1 molar hydrochloric acid and the solution is transferred with the use of not more than 1.5 ml. of water to a microbeaker which has been weighed together with its filter stick. Methyl red is added for an indicator, and ammonia is added until the color of the solution changes to yellow. The red coloration is then just restored by adding small portions of dilute hydrochloric acid; any precipitate formed by the ammonia will dissolve. The solution is treated with 0.1 gram of solid ammonium chloride, and water is added to make the volume equal to 3 ml. While the solution is heated on the steam bath, a 10% solution of diammonium hydrogen phosphate is slowly added. The total volume of the reagent is chosen so that 0.2 ml. is added for each milligram of zinc present.

The mixture is then heated for 15 minutes on the steam bath, and by that time the originally gelatinous precipitate will have become crystalline. After heating for 15 more minutes, the beaker is taken from the steam bath and its contents are allowed to cool to room temperature. The solution is drawn off through the filter stick, and the precipitate is washed with four 1-ml. portions of hot 1% diammonium hydrogen phosphate solution and then with four 1-ml. portions of cold water. The precipitate is dried for 1 hour at 100° to 105° C. in the drying block (*3*), and weighed as $ZnNH_4PO_4$.

Isolation of Aluminum and Iron. The combined filtrates *F*6 and *F*7, are evaporated to a volume of 1 to 2 ml., and bromine is added to oxidize all the iron to the ferric state. The bromine vapor is allowed to flow from the bottle into the microbeaker, the contents of the latter being swirled from time to time, until an excess of bromine is indicated by the color of the solution. The beaker is then placed on the steam bath and a stream of air, which has been freed from carbon dioxide, is blown over the solution until the bromine is completely removed. Methyl red, 0.3 ml. of 12 molar hydrochloric acid, and a small amount of paper pulp are added to the solution, which is diluted with water to a volume of 5 ml. The hot solution is treated with ammonia until the color of the indicator changes to yellow.

The precipitate of hydrated oxides is allowed to settle, whereupon the solution is removed through a filter stick with paper mat. The precipitate is washed with 1 ml. of a hot 2% solution of ammonium chloride. Filtrate *F*8 and washings are evaporated on the steam bath to a volume of 1 ml., while precipitate *P*8 is dissolved in 0.6 ml. of 6 molar hydrochloric acid. The solution is sucked through the filter stick into another microbeaker, and the transfer is made complete by washing with 4.5 ml. of hot water. The solution is precipitated with ammonia, and the hydrated oxides are separated from the solution as directed above. The filtrate and washings, *F*9, are combined with the concentrate of filtrate *F*8, and the combined solutions are immediately evaporated to a volume of 4 ml. and reserved for the determination of calcium and magnesium.

Separation and Determination of Iron and Aluminum. Two milliliters of 2.5 molar hydrochloric acid are introduced into the beaker containing the filter stick with the washed hydrated oxides, *P*9, and the beaker is heated on the steam bath until the precipitate appears to be completely dissolved. The solution of *P*9 is then transferred through the filter stick into another microbeaker, and the transfer is made quantitative by washing with hot 2.5 molar hydrochloric acid. The paper mat of the filter stick, which has been used in the two filtrations of the hydrated oxides, and the paper pulp are ashed in a 3-ml. platinum crucible. If there is any discernible amount of ash left behind, it is fused with the smallest practical quantity of sodium carbonate. The melt is dissolved in water and dilute hydrochloric acid, and the solution obtained is added to the main portion of the solution of precipitate *P*9.

The solution of the hydrated oxides, *P*9, is evaporated to a volume of approximately 0.5 ml., so as to remove most of the hydrochloric acid, then diluted with water to a volume of 2 ml. and treated with 0.1 to 0.2 gram of tartaric acid. The beaker is placed on the steam bath to complete solution of the added tartaric acid, but is afterwards again cooled to room temperature. The cold solution is saturated with hydrogen sulfide, treated with an excess of ammonia (1 ml. of 12 molar ammonia will suffice), and again saturated with hydrogen sulfide. One more drop of ammonia solution is added, and then the precipitate is allowed to settle. The capillary supplying the hydrogen sulfide is rinsed with water which has been saturated with the gas. A filter stick with a mat of fritted glass is used for separating the solution from the sulfide precipitate. Filtrate *F*10 and washings are collected in a microbeaker which has been weighed together with a porcelain filter stick provided with a filter disk of porous porcelain (*3*). The sulfide precipitate is washed with 2 ml. of a solution containing 3% ammonium tartrate and some ammonium sulfide.

Aluminum is determined in filtrate *F*10 without delay. Ammonia is added to the solution until its odor is distinctly perceptible. The microbeaker with the solution is then placed on the steam bath, and while its contents are kept in swirling motion, 0.7 ml. of oxine reagent for each milligram of aluminum is slowly added. The reagent is prepared by dissolving 0.1 gram of 8-hydroxyquinoline in 0.3 gram of glacial acetic acid and then diluting with 2 ml. of water. In order to prevent separation of the 8-hydroxyquinoline from the solution, the contents of the microbeaker are again treated with a few drops of 6 molar ammonia, so that the odor is strongly perceived. Heating on the steam bath is continued for 10 minutes. The contents of the beaker are treated with a few more drops of 6 molar ammonia, and the solution is drawn off through the filter stick, while it is still hot. The precipitate is washed with six 0.5-ml. portions of hot water, dried at 130° C. in the drying block (*3*), and weighed after cooling.

The precipitate of iron sulfide, *P*10, is dissolved in 1 ml. of 3 molar hydrochloric acid, which is first used to dissolve the ferrous sulfide adhering to the capillary supplying the hydrogen sulfide and to rinse it into the microbeaker containing the filter stick with the main portion of the precipitate. The beaker containing the filter stick and the acid is briefly heated on the steam bath, and its contents are treated with 1 drop of 3% hydrogen peroxide. The solution is finally transferred through the filter stick to a microbeaker which has been weighed together with a porcelain filter stick. The transfer is made complete by washing with hot 3 molar hydrochloric acid.

Determination of the iron requires removal of the excess of acid. Thus, the solution containing the iron is evaporated to a volume of a few tenths of a milliliter. The remaining liquid is treated with small portions of 2 molar ammonia until the precipitate of hydrated ferric oxide no longer redissolves on mixing. The precipitate is brought into solution by adding 1 drop of 3 molar hydrochloric acid. The microbeaker is then placed in a water bath which has a temperature of 60° C., and, while the contents are kept in a swirling motion, the needed amount of oxine reagent

is slowly added; 0.5 ml. of this solution is required for 1 mg. of iron. While the temperature of 60° C. is maintained, 0.7 to 1.0 ml. of 50% ammonium acetate solution, which has been made neutral to methyl red, is added to the contents of the beaker in the water bath. If more than 2 mg. of iron are present, from 1.0 to 1.5 ml. of the acetate solution are added. The beaker with the precipitate is left for 10 more minutes in the water bath. After removal, the beaker and its contents are allowed to cool for 5 minutes, the solution is drawn off through the filter stick, and the precipitate is washed with four 0.5-ml. portions of hot water and then dried at 120° to 130° C. in the drying block (*3*).

Determination of Calcium. The microbeaker containing combined filtrates *F*8 and *F*9 is placed on the steam bath, and its contents are treated with methyl red and 0.5 ml. of a 3% oxalic acid solution. Six molar ammonia is added slowly to the hot solution, which is set in swirling motion from time to time, until the color of the indicator changes to yellow. The solution with the precipitate is heated for 5 to 10 more minutes on the steam bath, then set aside for 1 hour, and allowed to cool to room temperature. The filtrate, *F*11, is removed through the filter stick, and the calcium oxalate is washed with three to four 0.5-ml. portions of cold 0.4% solution of ammonium oxalate and with two 0.3-ml. portions of cold water.

The calcium oxalate is dissolved by heating on the steam bath with 0.5 ml. of 3 molar hydrochloric acid. The solution is transferred through the filter stick to a microbeaker which has been weighed together with a suitable filter stick. The transfer is made complete by washing with 2 to 2.5 ml. of hot water. The calcium is again precipitated as described above. The solution is drawn off through the filter stick, and the precipitate is washed as before. Filtrate and washings are combined with filtrate *F*11. The precipitate is dried at 105° to 110° C. in a drying block and weighed as calcium oxalate monohydrate.

Determination of Magnesium. Filtrate *F*11 is evaporated to dryness. The residue is treated with 2 ml. of 14.5 molar nitric acid and heated on the steam bath after covering the opening of the microbeaker. When the evolution of gas ceases, the cover is rinsed into the beaker and removed. The contents of the beaker are again evaporated to dryness. The residue is now treated with 1 or 2 drops of 18 molar sulfuric acid (sp. gr. 1.84), the excess of which is driven off by heating while blowing a stream of air into the microbeaker. The residue is dissolved by heating on the steam bath with 1 drop of 3 molar hydrochloric acid and 0.5 ml. of water. The solution is transferred through a filter stick into a microbeaker which has been weighed together with a suitable filter stick. The transfer is made complete by washing with 1 to 1.5 ml. of hot water. Carbon and insoluble silica remain on the filter stick and are discarded.

The clear extract, *E*12, is treated with methyl red and then with small portions of 2 molar ammonia until the color of the solution changes to yellow. After addition of 0.2 ml. of 3 molar ammonium chloride (15% solution) the contents of the beaker are heated to the temperature of the steam bath, then 0.1 ml. of 6 molar ammonia and 0.1 ml of a 0.3 molar solution of secondary sodium or ammonium phosphate are quickly added, and the contents of the beaker are mixed by swirling and allowed to stand at room temperature for not less than 6 hours. Then the solution is drawn off through the filter stick, and the precipitate is washed with four 1-ml. portions of 1 molar ammonia and finally with two 0.5-ml. portions of methanol or ethanol. The precipitate is dried at room temperature by exposing it for 10 minutes to a current of air which has been passed through a tower containing calcium chloride hexahydrate, and is weighed as magnesium ammonium phosphate hexahydrate. For a control the precipitate is dissolved in hydrochloric acid and again precipitated from the solution and weighed.

Discussion. The gravimetric methods for the determination of the various elements have been taken from the microchemical literature (*13*). The scheme of separation in general follows established macroprocedures, and has been tested by applying it to a solution that contained known amounts of the constituents in question (Table III).

Table III. Application of Scheme to Synthetic Solution

Constituent	Present	Found	Error
	Mg.	*Mg.*	*Mg.*
SiO_2	0.000	0.000	0.000
Cu	0.184	0.187	+0.003
Zn	5.456	5.494	+0.038
Al	0.061	0.064	+0.003
Fe	0.522	0.548	+0.026
CaO	0.058	0.063	+0.005
MgO	0.047	0.055	+0.008

Some particulars of the procedure seem to call for explanation. It is preferable to dissolve the sample, evaporate the solution, and dehydrate the silica in the same platinum crucible in which the silica is collected, ignited, and weighed. This not only eliminates the undesirable transfer of the silica, but reduces the amount of filter paper needed, and, consequently, the size of the blank. This procedure could have been followed in the analysis of a pure sphalerite which could be decomposed by hydrochloric acid. The ore samples, however, contained traces of arsenic and were liable to be mechanically contaminated by small amounts of pyrites. The ignition of an insoluble residue containing these substances may easily spoil a platinum crucible. The ore samples are completely decomposed by nitric acid, but large amounts of sulfur are formed, which again render hazardous the ignition in a platinum crucible.

The hydrogen sulfide group is precipitated from a solution which is 0.6 molar with respect to hydrochloric acid. All the bismuth and most of the cadmium precipitate as sulfides, but almost all lead will remain in solution and will precipitate with the zinc sulfide and, subsequently, with the zinc ammonium phosphate. Presumably the lead will then be weighed as $Pb_3(PO_4)_2$ together with the zinc ammonium phosphate, and calculation shows that the zinc content will be found 0.25% too high, if 0.50% of lead is contained in the analyzed sample. The correctness of this reasoning has been tested by dissolving 0.82 mg. of lead and 12.43 mg. of zinc in nitric acid and precipitating the solution with ammonium phosphate. The weight of the precipitate was found equal to 34.96 mg., which is in satisfactory agreement with the calculated weight of 35.00 mg. Obviously, determination of the zinc by weighing the zinc ammonium phosphate is permissible only if the lead content is low. Whenever the amount of lead in the ore exceeds 0.5%, the zinc should be precipitated as 8-hydroxyquinolate, which permits a quantitative separation from the lead as reported by Cimerman and Wenger (*8*).

The procedure is able to take care of only small amounts of bismuth as found in the sphalerite under investigation. Large amounts of bismuth would for the most part remain insoluble when the residue from evaporation is extracted with 5 cu. mm. of nitric acid and 0.5 ml. of hot water previous to the titrimetric determination of copper.

A photoelectric colorimeter, Brunius type, was used for the determination of bismuth (*11*). Without the addition of glycerol to the solution and with the use of Zeiss filter S 47 (470 mμ) it was observed that Beer-Lambert's law holds up to 0.025 mg. of bismuth per ml. For a control, 0.4 mg. of copper and 0.1 mg. of bismuth were precipitated with hydrogen sulfide as described in the procedure. After titrimetric determination of the copper, the solution was diluted to 10 ml., and the extinction was determined. It corresponded to a total of 0.09 mg. of bismuth in the sample. For an appraisal of the sensitivity of the colorimetric method it may be added that the coloration produced by 0.01 mg. of bismuth can be clearly seen by the eye.

Use of the ammonium hydroxide separation for the trivalent metals of the third group may appear inviting, since only small amounts of these metals are present. Nevertheless, the small amount of zinc, present in the hydrated oxides even after double precipita ion, would accompany the iron and increase the percentage of iron found to an extent which appears undesirable because of the relatively small amount of iron present. Thus, isolation of the zinc by precipitation of the sulfide previous to ammonia separation of the trivalent ions was chosen. Precipitation of zinc sulfide from a bisulfate buffer solution (*11*, *20*, *24*, *26*) was selected in order to obtain a crystalline precipitate which is easy to filter. An additional advantage of this scheme is that double precipitation isolates the ammonium hydroxide group in sufficient purity in the absence of zinc. The necessity of triple precipitation would increase the risk of losing aluminum.

The zinc is weighed as the zinc ammonium phosphate rather than as the 8-hydroxyquinolate, for the latter precipitate is rather voluminous and presents difficulties in handling because of the large quantity of zinc in the samples. In one trial 7.315 mg. of pure metallic zinc were dissolved in hydrochloric acid and precipitated as zinc ammonium phosphate. The weight of the precipitate corresponded to 7.355 mg. of zinc. In another experiment 4.863 mg. of metallic zinc and 0.326 mg. of iron were dissolved. Separation of the zinc sulfide, dissolution, and precipitation of the zinc ammonium phosphate as outlined in the procedure gave a recovery of 4.871 mg. of zinc.

Conversion of the zinc sulfide to the oxide by roasting and weighing of the latter may appear simpler than the proposed procedure. There are, however, not less than three serious disadvantages connected with the use of zinc oxide as a weighing form. It contains somewhat more than 80% of zinc, and weighing errors, which have to be considered in microanalysis, are propagated with nearly full force into the calculated percentages. The zinc oxide is hygroscopic (*15*). Finally, complete conversion to the oxide requires a temperature of not less than 935° C. (*6*), and it always is preferable in microwork to avoid the use of high ignition temperatures.

After separation from the aluminum the iron may be reduced with stannous chloride and titrated with permanganate (*19*). Any zinc attending the iron will be without effect. The results of the titrimetric determination of iron are in good agreement with those obtained by weighing the 8-hydroxyquinolate, if the reduction with stannous chloride is performed with the proper care. The gravimetric determination, however, is less time-consuming.

DETERMINATION OF SULFUR, PHOSPHORUS PENTOXIDE, AND MANGANOUS OXIDE

Solution of Sample. Approximately 5 mg. of the sphalerite are weighed exactly in a microbeaker of 5-ml. capacity and dissolved by the use of either of the following two methods:

Procedure 1. The ore is treated with 0.5 ml. of bromine water and the mixture is allowed to stand for 15 minutes. One volume of concentrated hydrochloric acid is mixed with 3 volumes of concentrated nitric acid, and the liquid is shaken with an excess of bromine. One milliliter of this solution of bromine in nitric-hydrochloric acid is then introduced into the microbeaker, which is covered and allowed to stand overnight. The next day the contents of the beaker are inspected, and if any sulfur seems to have separated, some fuming nitric acid is added.

Procedure 2. A bromine–potassium bromide solution is prepared by adding 10 ml. of bromine, which is free from sulfur, to a saturated aqueous solution of 16 grams of potassium bromide and then diluting with water to 100 ml. The sample of sphalerite is treated with 0.4 ml. of this reagent and allowed to stand for 1 hour. Then 0.6 ml. of 14.5 molar nitric acid is added. The beaker is covered and allowed to stand overnight.

Whichever procedure is followed, the covered microbeaker with the digest of the ore is finally placed on the steam bath. When the evolution of gas has ceased, the cover is rinsed into the beaker and removed, and the contents of the microbeaker are evaporated to dryness. The residue is treated with 0.5 ml. of 7.3 molar hydrochloric acid, and the mixture is evaporated to dryness. The evaporation with the hydrochloric acid is repeated once. The residue is heated for 30 minutes at 105° C. It is then briefly heated with 0.1 ml. of 7.3 molar hydrochloric acid and 1 ml. of water, whereupon the extract is transferred through a filter stick with paper mat to a microbeaker which has been weighed together with a porcelain filter stick. The transfer is made complete by washing with 1 ml. of 0.6 molar hydrochloric acid and finally with approximately 3 ml. of hot water.

The insoluble residue, *R*1, is transferred to a platinum crucible and treated as in the procedure for the determination of the metallic constituents. It is evaporated with hydrofluoric and sulfuric acids, fused with sodium carbonate, and finally dissolved in hydrochloric acid.

Determination of Sulfur. Filtrate *F*1 has a volume of approximately 5 ml. and contains 0.6 millimole of hydrogen chloride. It is treated with 0.1 ml. of a solution containing 2 grams of hydroxylamine hydrochloride and 10 grams of ammonium chloride in a volume of 1 liter. The microbeaker is placed on the steam bath, and 0.2 ml. of a hot 10% solution of barium chloride dihydrate is added quickly to hot filtrate *F*1. This volume of reagent will take care of 2.5 mg. of sulfur in the sample. The beaker is left for 5 more minutes on the steam bath, and its contents are then allowed to digest at room temperature for at least 1 hour, and preferably for 4 hours. Filtrate *F*3 is collected, together with the washings resulting from the application of 1 ml. of cold water. The rest of the washings obtained from three 1-ml. portions of hot water and two 0.3-ml. portions of ethanol are rejected. The precipitate is dried at room temperature in a current of air which has been passed through calcium chloride. For a control it may be dried at 150° C., and it should not change weight. The factor 0.1373 (sulfur over barium sulfate) is to be multiplied by 0.991 if method 1 is used in dissolving the sample, and by 0.956 if procedure 2 is followed. Thorough washing with water prepares the filter stick for another sulfur determination. If it is to be used for a different purpose, however, it is cleaned by sucking through it warm concentrated sulfuric acid and then rinsing with hot water.

Determination of Phosphorus Pentoxide. Filtrate *F*3 is heated on the steam bath and treated with an excess of sulfuric acid for elimination of the barium ion. Filtrate *F*4 is combined with the solution of residue *R*2 (Table IV) and then evaporated to the appearance of moderate fumes of sulfur trioxide. After cooling to room temperature, nitric acid is added for the oxidation of the hydroxylamine and the iron, and the mixture is again evaporated to fumes of sulfur trioxide. The treatment with nitric acid and evaporation is repeated once. The residue is dissolved in 1 ml. of 1.5 molar nitric acid. The solution is treated with 50 mg. of solid ammonium nitrate, and the phosphate is precipitated at 70° C. by adding 1 ml. of the molybdate reagent of Biltz (*5*). If a precipitate separates within 4 hours, it is converted to the magnesium ammonium phosphate (*23*).

Determination of Manganous Oxide. Filtrate *F*5 is collected without the washings and treated with 0.5 ml. of 14.5 molar nitric acid. The colorimetric determination of manganese follow

Table IV. Scheme for Determination of Sulfur, Phosphorus Pentoxide, and Manganous Oxide

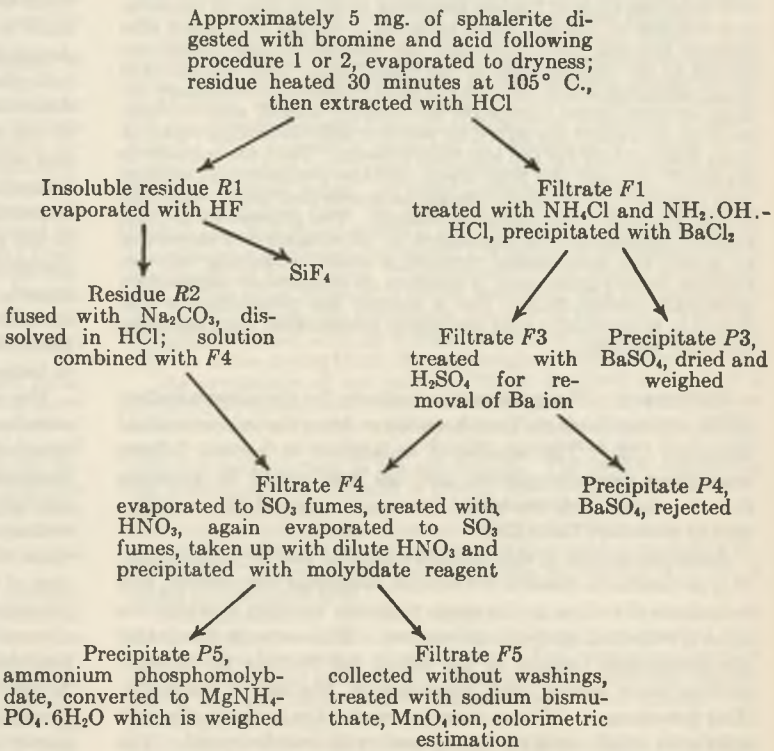


Table V. Determination of Correction Factor for Estimation of Sulfur

Alternate Procedure	Taken: Mohr's salt	Taken: Sulfur	Sulfur Obtained (= 0.1373 × $BaSO_4$)		Correction Factor
	Mg.	*Mg.*	*Mg.*		
1	5.865	0.9671	0.9791		0.9877
	11.121	1.8337	1.8456		0.9936
	28.931	4.770	4.797		0.9944
	10.507	1.7324	1.7547		0.9873
				Mean	0.9908
2	10.2796	1.6950	1.7739		0.9555
	10.2178	1.6848	1.7660		0.9540
	6.8270	1.1257	1.1745		0.9585
				Mean	0.9560

the directions of Hillebrand and Lundell (*15*). The cold solution is agitated with 50 mg. of sodium bismuthate for 1 minute, diluted with an equal volume of cold water, and filtered through a filter stick provided with a disk of fritted glass. The separation from the solid is made complete by washing with 0.5 molar nitric acid. A photoelectric colorimeter is used for estimation of the manganese in the filtrate. Large quantities of manganese are titrated (*15*).

Discussion. Since accurate determination of the sulfur in the sphalerite was required, careful design and investigation of the procedure to be used were necessary. Difficulties arise in solution of the sample. Fusion with sodium carbonate and potassium nitrate as in the micromethods of Treje and Alber (*25*) and Hecht (*12*) did not seem advisable, as macrowork indicates that it is difficult to obtain complete oxidation of the zinc sulfide in this manner. The presence of arsenic and lead in the material under investigation made undesirable the use of a platinum crucible, and, finally, it was realized that the necessary determination of the blank would be tedious and difficult. Fusion with sodium peroxide and some sodium carbonate is recommended for determination of sulfur in zinc blende (*7*). It seemed difficult, however, to avoid losses during the fusion, and special crucibles would be needed for performance on a microscale.

It was decided to try the customary wet methods for the oxidation of sulfidic ores. Aqua regia, which is used with pyrite, failed. Hydrogen sulfide is liberated from the sphalerite so rapidly that it partly escapes oxidation. Treatment with nitric-hydrochloric acid to which bromine is added is recommended for macroanalysis (*7*). Small samples, however, are too rapidly decomposed. A modification of this procedure, described as alternate procedure 1, proved satisfactory but for the fact that free sulfur is formed at times, which is rather difficult to oxidize and is easily overlooked. Therefore, the procedure described by Scott (*11*) was adapted to the micro scale (alternate procedure 2). The oxidation is slow but complete. The large amount of potassium salt, which is introduced with the reagent, must be taken into consideration when the sulfur is determined in the digest obtained.

The macromethod of Winkler (*27*) was adapted for microdetermination of the sulfur, since it permits the use of glass beakers, for the barium sulfate is dried at room temperature. Before weighing, the microbeaker with the barium sulfate was allowed to stand in the balance room for 25 minutes. The weight did not change when the beaker was left standing in the room for 30 minutes more and subsequent drying at 150° C. did not affect the weight. These findings support the statements of Schulek and Boldizsár (*21*) and indicate that Balarew's criticism (*1*) of Winkler's directions is not justified.

Because of the well-known variability of the composition of the barium sulfate precipitate, the empirical factors which are needed for obtaining a satisfactory degree of accuracy were determined by a series of controls. Ferrous ammonium sulfate hexahydrate, in which a sulfur content of 16.49% had been found by the customary macromethods, was used for a standard. The directions for the analysis of sphalerite were followed, and both alternate procedures of digestion were tried. The results are compiled in Table V. The theoretical factor, $S/BaSO_4 = 0.1373$, should be reduced by a correction factor equal to 0.9908, if procedure 1 is used for the oxidation of the sample, and by a factor of 0.9560, if procedure 2 is employed.

When Winkler investigated the accuracy of the determination of sulfur in pyrite, he also used ferrous ammonium sulfate hexahydrate for a standard (*28*). The barium sulfate was precipitated from a boiling solution which was 0.05 molar with respect to hydrogen chloride. The barium sulfate was dried at 132° C. and the theoretically calculated amounts of precipitate were obtained. The figures of Table V show that with procedures 1 and 2 the weights of the precipitates exceed the theoretically calculated amounts by 1 to 5%, respectively. Insufficient washing was not responsible, for the weights remained unchanged when the precipitates were washed with another 3 ml. of hot water. Obviously the conditions maintained by Winkler during the precipitation of the barium sulfate were not duplicated on the small scale. Neither was the solution agitated while adding the reagent, nor did heating on the steam bath give the same temperature as boiling over a flame. The effect of a deviation in the salt content of the precipitated solution is drastically demonstrated by the results obtained with alternate procedure 2. Nevertheless, procedures 1 and 2 give identical figures for the sulfur content, if the appropriate empirical factors are used. This is illustrated by Table VI, which lists the percentages of sulfur found in two samples of sphalerite which differed considerably in their quantitative composition.

The determination of manganese was tested by dissolving 0.0167 mg. of manganous oxide to which 10 mg. of Mohr's salt had been added, and then following the directions outlined. The colorimetric estimation indicated 0.017 mg. of MnO.

DETERMINATION OF LEAD AND CADMIUM

Procedure. From 2 to 8 mg. of sphalerite are accurately weighed in a microbeaker and dissolved by heating on the steam bath with a sufficient quantity of concentrated hydrochloric acid while the beaker is kept covered. When the reaction ceases, 0.01 ml. of concentrated nitric acid is added, and the heating is continued for a few more minutes. The cover is then rinsed into the beaker and removed, and the contents of the beaker are evaporated to dryness. The residue is treated with 0.5 ml. of 12 molar hydrochloric acid, and the mixture is briefly heated on the steam bath. The solution is transferred with the aid of a micropipet to a centrifuge cone of 1.5-ml. capacity, which is graduated at 0.1-ml. intervals. The transfer is made complete by rinsing microbeaker and pipet with hot water. The solution in the cone is evaporated to a volume of 0.1 ml., and is then diluted to a volume of 0.2 ml. by adding 7.5 molar hydrochloric acid.

The contents of the cone are treated with 10 mg. of pure aluminum. When this metal has completely dissolved, the contents of the cone are cooled to room temperature and diluted with water to a volume of 0.6 ml. After thorough mixing a suitable aliquot of the clear solution is transferred to the microcell of the polarograph, which is designed according to Majer (*18*) in the form of a small U-tube of 6-mm. bore, the bend of which is filled with the mercury serving for anode. The polarogram of the solution is recorded, 0.05 ml. of a solution of known lead and cadmium content is added, and again a polarogram is recorded. Using the standard calomel electrode as a reference, the wave of the lead is found at −0.45 volt and that of cadmium at −0.65 volt. The polarograms are evaluated as described by Kraus and Novak (*17*).

Discussion. The lead and cadmium contents of the sphalerite from Kristineberg were expected to be less than 0.5%, and it seemed desirable to be able to determine amounts as small as 0.05%. The macromethod for the determination of 0.2% of lead recommended by Scott (*11*) requires a 10-gram sample for titration of the lead with ammonium molybdate. Approximately the same amount of sample is required for gravimetric determination of the lead as sulfate. If either of these macromethods was

Table VI. Determination of Sulfur in Sphalerite

Sample No.[a]	Alternate Procedure Used	Sulfur Found, %[b]
1	1	32.76, 32.62 (31.45, 31.80)
	2	32.77, 32.76, 32.40
5	1	32.06, 31.75
	2	31.89, 31.70

[a] Complete composition of samples 1 and 5 given in Table VII.
[b] Low figures in parentheses explained by incomplete oxidation of sulfur.

Table VII. Analysis of Sphalerite from Kristineberg

	Sample No. 1	2	3	4	5	6
	Color of Pulverized Sample					
	Pale yellow	Pale yellow with gray tinge	Grayish yellow	Gray	Brown	Brown
SiO_2, %	0.95	0.1	0.9	1.20	1.57	1.15
Al_2O_3, %	<0.1	<0.1	<0.2	0.1	0.24	<0.2
MnO, %	0.02	<0.03	<0.03	0.05	0.07	0.09
MgO, %	0.15	0.1	<0.3	0.30	0.65	0.80
CaO, %	<0.2	<0.2	....	<0.2	0.25	<0.2
Cu, %	0.08	0.05	0.13	1.87	0.13	0.17
Cd, %	0.20	0.18	0.20	0.22	0.16	0.18
Pb, %	0.04	0.02	0.42	0.45	0.02	0.02
Bi, %	<0.05	<0.05	<0.1	<0.05	<0.05	<0.05
Fe, %	1.36	2.60	3.04	3.50	8.15	7.70
Zn, %	64.5	62.8	62.8	59.2	56.0	57.0
S, %	32.7	33.7	32.9	32.5	31.9	33.1
P_2O_5, %	<0.2	<0.2	....	<0.2	<0.2	<0.2
After applying corrections for presence of chalcopyrite, $CuFeS_2$, galena, PbS, talc, $H_2Mg_3Si_4O_{12}$, and chlorite, $H_8Mg_5Al_2Si_3O_{16}$						
Zn, %	65.46	63.31	63.68	64.43	58.33	58.40
Fe, %	1.30	2.58	2.97	2.03	8.38	7.73
S, %	33.05	33.92	33.15	33.30	33.19	33.69
Cd, %	0.20	0.18	0.20	0.24	0.17	0.18
Zn, mole	1.001	0.968	0.974	0.986	0.892	0.893
Fe, mole	0.023	0.046	0.053	0.036	0.150	0.138
S, mole	1.031	1.058	1.034	1.039	1.033	1.051
Cd, mole	0.002	0.002	0.002	0.002	0.002	0.002

to be used on a micro scale, at least 100 mg. of sphalerite would be required for a determination.

Hecht and Kroupa (*14*) describe a rather tedious micromethod for the gravimetric determination of approximately 0.1% of lead, determined as sulfate, in allanite. An electrolytic micromethod for the determination of 0.01% of lead in zinc has been described by Clarke and Hermance (*9*). Both methods, however, require 1 gram of sample.

Considering the small amounts of sphalerite available, an adaptation of the polarographic procedure of Kraus and Novak (*17*) for the determination of lead and cadmium in zinc ores appeared most inviting. These authors prepare 50 ml. of solution by starting with 1 gram of material. A hundredfold reduction of volume and mass appeared feasible and promised at the same time a suitable method for determining cadmium. Survey of the literature had shown the lack of any suitable micromethod for determining the small amounts of cadmium present in the sphalerite.

A solution containing 8 mg. of zinc, 1.3 mg. of copper, 0.2 mg. of iron, 0.114 mg. of cadmium, and 0.145 mg. of lead per milliliter was prepared. Controls were performed by analyzing 1-ml., 0.5-ml., and 0.1-ml. portions of this solution as directed. The heights of the lead and cadmium waves were identical with those observed with pure lead and cadmium solutions of the same concentrations.

CONCLUSIONS

The results of the analyses of six samples of sphalerite from Kristineberg are summarized in Table VII. The petrographic investigation of the Kristineberg ore indicates that pyrite, chalcopyrite, galena, talc, chlorite, and quartz may be present as inclusions in the sphalerite samples. The amounts of the last five minerals are indicated by the percentages found for copper, lead, alumina, magnesia, and silica. If the percentages of the constituents for which these minerals account are subtracted from the total, and the remaining percentages of zinc, iron, sulfur, and cadmium are recalculated to give a total of 100%, the figures of the lower portion of Table VII are obtained. The ratio of iron to magnesia in the chlorite of the Kristineberg mine is approximately 1 to 10. Thus, the iron content of any chlorite present need not be considered, since all the samples of sphalerite contain less than 1% of magnesia. Any talc present may also be considered free from iron.

If sphalerite is considered as zinc sulfide which contains variable quantities of ferrous sulfide and cadmium sulfide in solid solution, the deviation of the sulfur found from that calculated from the percentages of zinc, iron, and cadmium is for the 6 samples investigated: +0.16, +1.34, +0.16, +0.48, −0.35, and +0.57%, an average of +0.40%. The accuracy of the determinations of sulfur, zinc, iron, and copper is estimated as equal to ±0.25% sulfur, ±0.5% zinc, ±0.15% iron, and ±0.02% copper. Consequently, the analytical procedure may cause a discrepancy of ±0.6% between the determined and calculated sulfur contents. This permissible deviation is exceeded only in the instance of sphalerite sample 2, which was collected from a vein rich in pyrite and may contain grains of pyrite as a mechanical impurity. If sample 2 is excluded, the average difference between determined and calculated sulfur contents becomes ±0.2%. The findings seem to support those of Beutell and Matzke (*4*, *10*) who, in several determinations, obtained a zinc-sulfur ratio for sphalerite exactly equal to unity.

The iron content of the sphalerite of the Kristineberg mine, mainly marmatite, varies from 1 to 8.5%, corresponding to a variation in the zinc content from 65 to 58%, as may be seen from the lower part of Table VII. The amount of minute inclusions of pyrite, if present at all, is very small. Sample 6 was analyzed mainly to obtain a control of the analysis of sample 5 with which it is practically identical and which represents the darkest type of sphalerite found in the mine. Since the dark type of the mineral as represented by samples 5 and 6 is found only locally, the normal composition of the sphalerite from Kristineberg is approximately 64% zinc, 2.5% iron, 0.2% cadmium, and 33% sulfur.

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LITERATURE CITED

(1) Balarew, D., *Z. anal. Chem.*, 115, 104, 109 (1938).
(2) Benedetti-Pichler, A. A., IND. ENG. CHEM., ANAL. ED., 11, 226 (1939).
(3) Benedetti-Pichler, A. A., "Introduction to the Microtechnique of Inorganic Analysis", New York, John Wiley & Sons, 1942.
(4) Beutell, A., and Matzke, M., *Centr. Mineral. Geol.*, 1915, 263.
(5) Biltz, H., and Biltz, W., "Ausführung quantitativer Analysen", Leipzig, S. Hirzel, 1930.
(6) Bornemann, K., *Z. anorg. allgem. Chem.*, 82, 216 (1913).
(7) Chemiker-Fachausschuss des Metall und Erz e.V., "Analyse der Metalle, Vol. 1, Schiedsverfahren", Berlin, J. Springer, 1942.
(8) Cimerman, Ch., and Wenger, P., *Mikrochemie*, 24, 165 (1938).
(9) Clarke, B. L., and Hermance, H. W., *J. Am. Chem. Soc.*, 54, 877 (1932).
(10) Doelter C., "Handbuch der Mineralchemie", Vol. 4, Part 1, p. 313, Dresden and Leipzig, T. Steinkopf, 1912.
(11) Furman, N. H., "Scott's Standard Methods of Chemical Analysis", 5th ed., New York, D. Van Nostrand Co., 1939.
(12) Hecht, F., *Mikrochim. Acta*, 2, 202 (1937).
(13) Hecht, F., and Donau, J., "Anorganische Mikrogewichtsanalyse", Vienna, J. Springer, 1940.
(14) Hecht, F., and Kroupa, E., *Z. anorg. allgem. Chem.*, 236, 181 (1938).

(15) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis", New York, John Wiley & Sons, 1929.
(16) Hybbinette, A.-G., and Benedetti-Pichler, A. A., *Mikrochemie*, 30, 15 (1942).
(17) Kraus, R., and Novak, J., *Collection Czechoslov. Chem. Commun.*, 10, 543 (1938).
(18) Majer, V., *Mikrochemie*, 18, 74 (1935).
(19) Mika, J., "Die exakten Methoden der Mikromassanalyse", Stuttgart, F. Enke, 1939.
(20) Rieman, W., III, Neuss, J. D., and Naiman, B., "Quantitative Analysis", New York, McGraw-Hill Book Co., 1942.
(21) Schulek, E., and Boldizsár, I., *Z. anal. Chem.*, 120, 410 (1940).
(22) Schwarz-Bergkampf, E., *Mikrochemie, Emich-Festschrift*, 268 (1930).
(23) Thurnwald, H., and Benedetti-Pichler, A. A., *Z. anal. Chem.*, 86, 41 (1931).
(24) Treadwell, F. P., "Analytical Chemistry, Vol. II, Quantitative Analysis", 3rd ed., New York, John Wiley & Sons, 1914.
(25) Treje, R., and Alber, H., *Jernkontorets Ann.*, 9, 457 (1933).
(26) Weiss, G., "Ueber die quantitative Bestimmung und Trennung von Zink und Nickel", Munich, 1906.
(27) Winkler, L. W., *Z. angew. Chem.*, 30, I, 259 (1917).
(28) *Ibid.*, 33, I, 59, 159, 162 (1920).

Application of Colorimetry to the Analysis of Corrosion-Resistant Steels

Determination of Zinc

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A photometric method for the determination of zinc in corrosion-resistant steels by the dithizone method is presented. A General Electric spectrophotometer with a slit width of 10 millimicrons was used in developing the method, but it has also been adapted to the use of a Klett-Summerson photoelectric colorimeter using a Klett No. 52 green filter. The method is suited to routine use and 11 samples and a blank can be run daily using two double-funnel racks. By careful attention to details, accurate values can be obtained.

THERE is at present little, if any, published material on simple and accurate methods for the determination of small amounts of zinc in corrosion-resistant steels. The determination of this element was, therefore, included in an investigation of several residual elements by this laboratory (*2*, *12*, *16*).

Precipitation of zinc as sulfide from a weakly acid solution, suggested by Ibbotson (*9*), and completion as oxide have become a standard technique, successfully adapted to plain and low-alloy steels by Bright (*3*). This method, however, has not yielded satisfactory results with corrosion-resistant steels.

As the range to be covered is suited to colorimetric determinations, procedures employing dithizone as the color reagent were tested. While an extensive investigation of dithizone possibilities has been made by others, application of the procedure has been largely confined to solutions prepared from biological material (*7*), foods (*8*), and soils (*15*). These ordinarily contain such mixtures of elements that separations by pH control are usually sufficient. The dithizone method has also been adopted to Babbitt metal (*10*) and aluminum alloys (*6*).

DISCUSSION

Attempts to determine the zinc by precipitation as sulfide (*9*) were unsuccessful; zinc added to the solutions of corrosion-resistant steels was not recovered. Sulfide precipitates were obtained, filtered, and ignited, but purification of the residue by mercuric thiocyanate gave erratic results and in some instances yielded no precipitate. Investigation of the ignited precipitate by spectrographic analysis disclosed the presence of nickel but no trace of zinc. Subsequent qualitative analysis employing dithizone also failed to detect zinc in the sulfide precipitate. The method was, therefore, discarded.

All subsequent attempts were limited to dithizone extraction methods. As no dithizone procedures, directly applicable to steels, were known to exist, it was necessary to test the variety of suggestions that have appeared and combine promising steps to obtain a satisfactory method.

The sample is dissolved in a nitric-hydrochloric acid mixture, and copper is separated by hydrogen sulfide. This initial separation is unnecessary, except in unusual instances where the copper content of the alloy is relatively high. The chromium and tin are oxidized by use of perchloric acid and volatilized with dry hydrochloric acid gas. The solution is adjusted to approximately pH 8 with ammonium hydroxide after the addition of sufficient citric acid to prevent precipitation of the iron. A preliminary dithizone-chloroform extraction is performed which removes copper, zinc, and lead quantitatively, accompanied by nickel and cobalt. The zinc and lead are quantitatively removed from the dithizone by a weak acid extraction which effects an almost complete separation (*13*). The final extraction is made with dithizone–carbon tetrachloride in a buffered solution of pH 6.0 containing thiosulfate. The lead and traces of any other impurities remaining are thereby removed (*14*). The zinc can be determined by mixed colors (*4*), titrimetrically (*10*, *13*), or by removing excess dithizone with sodium sulfide solution and completing as a single color on a photoelectric colorimeter (*15*). The last method employing sodium sulfide has the advantage of being the means of effecting a further purification if impurities have passed through the previous steps (*5*).

The various methods tested which did not give consistent or satisfactory results are outlined below.

Attempts to obtain a separation of zinc by chloroform extraction in ammoniacal citrate solutions were unsuccessful. This method included a purification of the dithizone solution by acid extraction and a second chloroform-dithizone shake-out. If the hydroxyl-ion concentration was raised above pH 8.5 the nickel interfered seriously. Removal of the iron and a large portion of the nickel was attempted with ammonium oxalate in ammoniacal solution (*11*). This preliminary step was combined with the above procedure, but results were not consistent. As lead undoubtedly interfered, its removal was undertaken. Holland and Ritchie (*8*) employed sodium diethyl dithiocarbamate to prevent reaction between dithizone and lead. Tests of this procedure with pure solutions disclosed that it was entirely empirical and zinc is partially and in some cases completely removed. Such conditions as length of time the aqueous solution remained in contact with the dithizone-chloroform solution,

Table I. Analytical Data

Sample	Zinc Added %	Zinc Found[a] %	Sample	Zinc Added %	Zinc Found[a] %
Blank	...	0.003	20% Cr, 10% Ni steel	0.010	0.016
18% Cr, 8% Ni steel	...	0.002			0.014
		0.002	20% Cr, 10% Ni steel	0.015	0.021
		0.002			0.021
		0.002	20% Cr, 10% Ni steel	0.020	0.026
18% Cr, 8% Ni steel	0.005	0.004			0.024
		0.005	Blank	...	0.002
18% Cr, 8% Ni steel	0.010	0.011			
		0.011	25% Cr, 20% Ni steel	...	0.010
18% Cr, 8% Ni steel	0.015	0.017			0.010
		0.015			0.010
18% Cr, 8% Ni steel	0.020	0.021			0.012
		0.019	25% Cr, 20% Ni steel	0.005	0.014
Blank	...	0.002			0.014
			25% Cr, 20% Ni steel	0.010	0.021
20% Cr, 10% Ni steel	...	0.005			0.019
		0.006	25% Cr, 20% Ni steel	0.015	0.024
		0.006			0.025
		0.006			
20% Cr, 10% Ni steel	0.005	0.011	25% Cr, 20% Ni steel	0.020	0.032
		0.010			0.030

[a] Results for samples have been corrected for blank.

length of mixing, and vigor of shaking introduced variable factors. Cowling and Miller (*4*) have stated that consistent, although empirical, results are obtained with the use of the dithiocarbamate for the separation of lead from zinc. Attempts to use this reagent for routine analysis were abandoned. A method submitted for trial (*1*) made use of two dithizone-chloroform extractions, the first from an ammoniacal solution and the second, following an acid separation of copper, from a solution with pH about 5. Following the above procedure, the final extraction was found to be empirical and difficulty was experienced by different operators in obtaining consistent results.

Experiments established that chloroform was superior to carbon tetrachloride as solvent for dithizone for removing zinc from ammoniacal solutions. Use of the latter solvent caused a large increase in the amounts of unwanted elements extracted, notably nickel, and extraction of the zinc was slow. On the other hand, in acid solution, the use of chloroform as solvent definitely gave less desirable results. The employment of the two solvents for extraction, under their most favorable pH conditions and in conjunction with each other, yielded satisfactory results which were shown to be quantitative (Table I).

A spectrophotometric study was made to investigate the feasibility of the resolution of the mixed colors. Figure 1 discloses measurements taken in the region of maximum absorption by the zinc dithizonate (approximately 535 mμ), including absorption of the reagent. The zinc dithizonate complex displays only a single absorption maximum (see curve 2 of Figure 1). Attempts to determine the excess dithizone at 615 mμ were more successful. Interference caused by the zinc dithizonate is negligible. However, the amounts of dithizone solution must be carefully controlled and kept constant.

Several methods have been recommended for removal of the uncombined dithizone (*7*). The use of a dilute solution of sodium sulfide was found satisfactory. In addition to removal of the free dithizone, interfering elements such as cadmium, stannous tin, and lead are also extracted from the carbon tetrachloride layer (*5*). Measurements made on zinc dithizonate solutions, which were obtained by subjecting pure salt solutions to the procedure adopted, displayed adherence to Beer's law. Beer's law was also shown to be valid for solutions of a corrosion-resistant steel containing varying amounts of zinc.

The actual region used for determining the zinc was at 520 mμ. A slight shift from the maximum absorption peak was made to decrease possible interference by dithizone.

APPARATUS

A spectrophotometer or photoelectric colorimeter may be used (a General Electric recording spectrophotometer and a Klett-Summerson photoelectric colorimeter were used by the authors). Readings are made at 520 millimicrons, to minimize interference by dithizone.

Pyrex ware is thoroughly washed with concentrated hydrochloric acid and rinsed with double-distilled water.

REAGENTS

Zinc-free distilled water, obtained by redistilling ordinary distilled water in an all-Pyrex still.

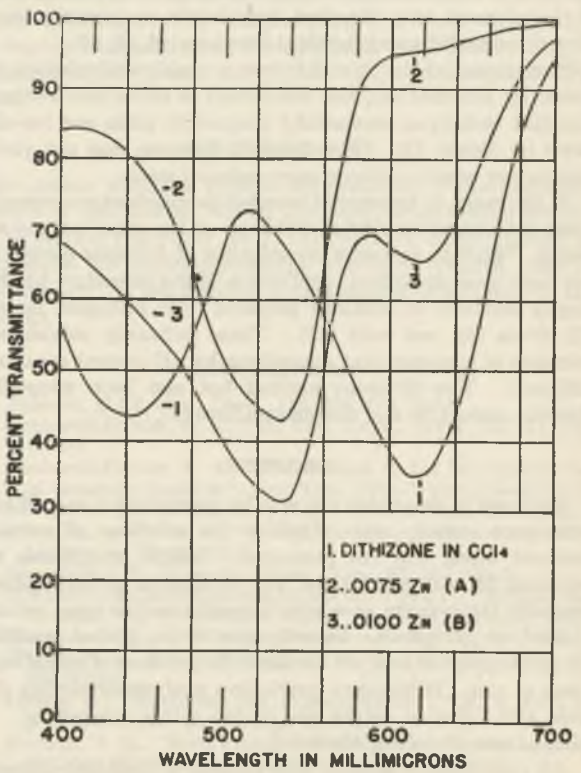

Figure 1. Transmission of Zinc-Dithizone System

A. Excess dithizone removed, volume 25 ml.
B. Excess dithizone present, volume 25 ml. All recordings made using cell depth of 0.25 cm.

STANDARD ZINC CHLORIDE SOLUTION. Transfer exactly 1.000 gram of National Bureau of Standards zinc metal 43e to a 1000-ml. volumetric flask, add 10 ml. of hydrochloric acid (1 to 1), and warm until zinc is completely dissolved. Cool and dilute to the mark with zinc-free distilled water at 20° C. Mix the solution thoroughly. Pipet 100 ml. of the solution into another 1000-ml. volumetric flask, dilute to mark with zinc-free distilled water at 20° C., and mix this solution thoroughly. Pipet 100 ml. of the diluted solution into another 1000-ml. volumetric flask and dilute to the mark with zinc-free distilled water at 20° C. One milliliter of this solution contains 0.01 mg. of zinc.

CITRIC ACID SOLUTION. Dissolve 20 grams of C.P. citric acid in 100 ml. of zinc-free distilled water.

HYDROCHLORIC ACID SOLUTION (0.1 *N*). Dilute 9 ml. of hydrochloric acid (sp. gr. 1.19) to 1000 ml. with zinc-free distilled water.

AMMONIUM HYDROXIDE SOLUTION (0.1 *N*). Dilute 8 ml. of ammonium hydroxide (sp. gr. 0.90) to 1000 ml. with zinc-free distilled water.

METHYL ORANGE INDICATOR SOLUTION. Dissolve 0.1 gram of methyl orange in 100 ml. of zinc-free distilled water.

SODIUM THIOSULFATE SOLUTION. Dissolve 15 grams of C.P. sodium thiosulfate in 100 ml. of zinc-free distilled water.

PHTHALATE BUFFER SOLUTION (pH 6.0). Add 79 ml. of 0.1 *N* sodium hydroxide to 2.000 grams of C.P. acid potassium phthalate in a 100-ml. volumetric flask and dilute to the mark with zinc-free distilled water.

CHLOROFORM-DITHIZONE SOLUTION. Dissolve 0.010 gram of diphenylthiocarbazone in 100 ml. of C.P. chloroform and store in a glass-stoppered brown bottle.

CARBON TETRACHLORIDE–DITHIZONE SOLUTION. Dissolve 0.010 gram of diphenylthiocarbazone in 500 ml. of C.P. carbon tetrachloride and store in a glass-stoppered, brown bottle.

SODIUM SULFIDE SOLUTION. Dissolve 0.05 gram of C.P. sodium sulfide in 100 ml. of zinc-free distilled water.

PROCEDURE

Transfer 0.05 gram of the sample to a 50-ml. Erlenmeyer flask, add 3 ml. of concentrated hydrochloric acid and 2 ml. of concentrated nitric acid, and warm until solution is complete. Add 3 ml. of 70% perchloric acid and 1 drop of 48% hydrofluoric acid, and place on a hot plate. Evaporate the solution until perchloric acid vapors condense in the neck of the flask and all the chromium is oxidized. Introduce a stream of dry hydrochloric acid gas into the flask to volatilize chromium and tin. Cool the flask and dilute with 10 ml. of zinc-free distilled water.

Add 5 ml. of citric acid solution, followed by sufficient concentrated ammonium hydroxide dropwise until the solution shows alkaline to litmus paper and then 2 to 3 drops in excess. The pH of the solution should be 8.0 to 8.5. Cool the solution to room temperature and transfer to a clean 125-ml. Squibb separatory funnel with a minimum amount of zinc-free distilled water.

Add 10 ml. of chloroform-dithizone solution to the separatory funnel and shake vigorously for about 20 seconds. Allow the chloroform layer to separate and withdraw it into another clean separatory funnel. A double funnel rack is convenient for having one set of funnels under another. Continue to extract the aqueous solution with 5-ml. portions of chloroform-dithizone until the dithizone is a greenish-purple after the shaking, withdrawing each successive chloroform layer into the separatory funnel containing the previous extractions. If the copper is not too high, over 0.50%, 20 to 30 ml. of chloroform-dithizone solution will be sufficient. All the zinc is now in solution in the chloroform as zinc dithizonate and, therefore, the aqueous solution can be discarded.

Add 10 ml. of 0.1 *N* hydrochloric acid to the chloroform solution and mix well for at least one minute. Allow the layers to separate, withdraw and discard the chloroform. Add 5 ml. of chloroform to the hydrochloric acid solution and mix to extract any remaining dithizone. Remove the chloroform and repeat the washing with one more 5-ml. portion of chloroform, discarding it also. Add 1 drop of methyl orange indicator to the hydrochloric acid solution and titrate with 0.1 *N* ammonium hydroxide until the indicator changes to a definite yellow color. Add 1 ml. of sodium thiosulfate solution and 5 ml. of phthalate buffer solution.

Add exactly 25 ml. of carbon tetrachloride–dithizone solution and shake for 1 minute, allow the layers to separate thoroughly, and remove the aqueous layer by means of a siphon. Add 25 ml. of sodium sulfide solution to the separatory funnel and shake for 15 seconds to remove the excess dithizone. When the layers have divided, siphon off the sulfide solution and shake out again with 25 ml. of sodium sulfide solution. After the layers have separated, drain a portion of the zinc dithizonate in carbon tetrachloride into a colorimeter cell and take a reading, using a Klett-Summerson photoelectric colorimeter with a Klett 52 green filter. Results are obtained by reference to a graph prepared from zinc standards. It is very important that a blank on reagents be carried through the procedure exactly as on the samples every time determinations are run, preferably using a steel sample known to be free of zinc.

Prepare standards by adding 10 ml. of zinc-free water, 1 ml. of 25% sodium acetate, and varying amounts of standard zinc chloride solution to clean dry funnels. Add 25 ml. of carbon tetrachloride–dithizone and complete as directed above. A straight-line curve is obtained.

NOTES ON THE METHOD

Cleanliness throughout the entire operation is of prime importance. Dust and fumes from foundries, furnaces, mills, etc., were found to have a very high percentage of zinc. Glassware, especially new, must be thoroughly cleaned. Chemicals used must be of the purest grade and if found necessary, by test, they should be purified by making slightly ammoniacal and extracting with dithizone-chloroform solution until the dithizone remains green, then filtering through a loose paper or pledget of cotton. The acids are used from fresh stock bottles unless found to give an excessively high blank; in that case, the acid should be redistilled. Exercising the greatest care, the blank can be kept under 2 micrograms of zinc.

If the copper is over 0.50% in the sample it is well to remove it by means of hydrogen sulfide to prevent the use of large amounts of dithizone. This is best accomplished by dissolving the sample in 5 ml. of concentrated hydrochloric acid and evaporating to approximately 1 ml. Add 15 ml. of zinc-free water and pass a stream of hydrogen sulfide over the surface of the solution, swirling occasionally, until saturated. Heat to boiling and let stand warm for 15 minutes. Filter on a small paper and wash with small amounts of zinc-free water into a 50-ml. Erlenmeyer flask. Add the perchloric acid and follow the regular procedure.

The dry hydrochloric acid gas, for volatilization of chromium and tin, is produced by allowing concentrated hydrochloric acid to drop into concentrated sulfuric acid, via a separatory funnel in a suction flask, and passing the gas over the surface of the solution on a very hot plate.

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LITERATURE CITED

(1) Boyer, W. J., Rustless Iron & Steel Co., Baltimore, Md., unpublished work.
(2) Bricker, L., and Proctor, K. L., IND. ENG. CHEM., ANAL. ED., 17, 511 (1945).
(3) Bright, H. A., *Bur. Standards J. Research*, 12, 383 (1934).
(4) Cowling, H., and Miller, E. J., IND. ENG. CHEM., ANAL. ED., 13, 145 (1941).
(5) Fischer, H., and Leopoldi, G., *Aluminium*, 25, 356 (1943).
(6) Fischer, H., and Leopoldi, G., *Chem.-Ztg.*, 64, 231 (1940).
(7) Hibbard, P. L., IND. ENG. CHEM., ANAL. ED., 9, 127 (1937).
(8) Holland, E. B., and Ritchie, W. S., *J. Assoc. Official Agr. Chem.*, 22, 333 (1939).
(9) Ibbotson, F., "Chemical Analysis of Steel Works' Materials", p. 229, London, Longmans, Green and Co., 1920.
(10) Kolodub, P. A., *Zavodskaya Lab.*, 9, 514 (1940).
(11) Lundell, G. E. F., and Hoffman, J. T., "Outlines of Methods of Chemical Analysis", p. 64, New York, John Wiley & Sons, 1938.
(12) Milner, O., Proctor, K. L., and Weinberg, S., IND. ENG. CHEM., ANAL. ED., 17, 142–5 (1945).
(13) Prodinger, W., "Organic Reagents Used in Quantitative Inorganic Analysis", p. 133, New York, Elsevier Publishing Co., 1940.
(14) Sandell, E. B., IND. ENG. CHEM., ANAL. ED., 9, 464 (1937).
(15) Sherman, G. P., and McHargue, J. S., *J. Assoc. Official Agr. Chem.*, 25, 510 (1942).
(16) Weinberg, S., Proctor, K. L., and Milner, O., IND. ENG. CHEM., ANAL. ED., 17, 419 (1945).

Fluorescent Bead Test for Uranium in Minerals

A Critical Study

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The fluorescent sodium fluoride bead test will detect 0.05 microgram of uranium in a single grain of sample. It is specific except for columbium, which gives a fainter fluorescence. Excess cerium or rare earths interfere. Both troubles are overcome in minerals containing over 1% of uranium by adjusting the ratio of sample to sodium fluoride. An ether extraction method for separating uranium from columbium, cerium, and the rare earths permits detection of 0.0001% of uranium in 0.5 gram of columbite or nonuraniferous cerium minerals. Excess silicon dioxide, titanium dioxide, etc., may suppress fluorescence, but can be removed by extra heating. About 1 mole of uranium in 2000 of sodium fluoride forms a fluorescent complex when fused. At low flame temperatures any excess remains undissolved. At higher temperatures excess is converted to sodium uranate.

IN 1926 Nichols and Slattery (*9*) noted that, when a trace of a uranium compound is fused with sodium fluoride on a platinum wire, the resulting bead has a vivid lemon yellow fluorescence under long-wave ultraviolet light. It was stated that maximum brilliance occurs at a concentration of about 1 mole of uranium in 2000 of sodium fluoride and that the fluorescence is still visible in 10,000,000 moles of sodium fluoride. Clearly, a reaction of this sensitivity would be very attractive for the detection of uranium in minerals, if sufficiently reliable.

A year later Papish and Hoag (*10*) proposed using this fluorescent bead as a test for uranium. However, they failed to make an adequate study of interfering elements, noting only that columbium pentoxide produced a misleading fluorescence in sodium fluoride, and that such compounds as columbium pentoxide, titanium dioxide, and silicon dioxide, if present in great excess might reduce the brilliance of uranium fluorescence by tying up all or part of the sodium fluoride. They were apparently unaware of a previous report by Nichols and Howes (*8*) regarding the production of fluorescence in sodium fluoride by neodymium and erbium.

Later Hernegger (*3*) mentioned that iron and manganese may cause trouble by coloring the bead, while thorium tends to reduce the strength of uranium fluorescence. Both Hernegger and Karlik (*3*) and Hoffmann (*4*) proposed the fluorescent sodium fluoride bead as the basis of micromethods for uranium determination, requiring laborious removal of all other elements. Although their work suggests that many elements may interfere for quantitative purposes, neither mentioned whether the degree of interference is sufficient to reduce the reliability of the method as a qualitative test.

In view of the lack of complete data as to what elements have been tested for the production of a misleading fluorescence, for the existence and extent of quenching of fluorescence, as well as for interference by reaction with the sodium fluoride, it seemed necessary to check all the elements capable of occurrence in minerals for the possible types of interference before accepting this method with full confidence. In addition, elements occurring together in a given mineral might react during fusion with each other, or with the sodium fluoride, or both, to form new interfering compounds. Since little has been published regarding the chemistry of reactions in molten sodium fluoride, the only alternative was to test a representative series of uranium minerals, particularly as prior workers reported testing only a very limited number. As there seem to have been no other significant contributions to this subject, the indicated work was undertaken.

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APPARATUS AND METHODS

It seemed likely that any fluorescence of elements other than uranium might be eliminated by using an argon bulb, which emits only long-wave ultraviolet (down to approximately 3100 Å.). This includes the wave lengths capable of exciting the fluorescence of uranium in sodium fluoride, whereas other sources may produce both long- and short-wave radiation. Short wave lengths are not necessary for this purpose and might excite interfering fluorescence in test beads which would be inert to the near ultraviolet. One such instance is mentioned below. A quartz-mercury lamp (R. & M.) with Corning No. 9860 filter produced a very faint orange fluorescence in control beads made from several samples of C.P. sodium fluoride, again showing the desirability of dispensing with all but the ultraviolet wave lengths necessary to excite uranium fluorescence.

It soon became apparent that a filter would be desirable in examining test beads with only a faint fluorescence. After several trials, a Corning No. 9860 dark red-purple polished filter of 3-mm. thickness was found most suitable. This also transmits shorter wave lengths than are emitted by the argon bulb, but is of optimum density to cut off most of the visible light without holding back more than a little of the weak long-wave ultraviolet available.

The method is similar to that used for any bead test, with the exception of the following precautions:

1. Since the test is exceedingly sensitive, it is always necessary to have the wire scrupulously clean and to test the sodium fluoride bead for fluorescence before addition of the sample.
2. Maximum brilliance of uranium fluorescence was obtained only when the bead was heated until completely fluid and as clear as possible after addition of the sample.
3. Fluorescence did not appear in test beads until they had cooled to a point only slightly above room temperature.
4. A test bead which is yellowish when cold may give the appearance of having a faint yellow fluorescence. Interposing the No. 9860 filter will distinguish between true fluorescence and a simple color effect, except in the case of extremely faint fluorescence. In that case examination of the bead with a hand spectroscope having a wide slit will be helpful. The fluorescence spectrum of uranium in sodium fluoride has a narrow band in the yellow-green which is visible even at very low intensity levels.
5. To avoid eye strain from the strong sodium flame and subsequent difficulty in dark adaptation, it is helpful to use a blue filter for observing the bead during fusion.

TESTS FOR INTERFERENCE BY OTHER ELEMENTS

Each of the elements listed below was added to a nonfluorescent sodium fluoride bead, and the bead was fused again, cooled, and examined for fluorescence. In most cases, additional portions of the element in question were added and the test was repeated to check the effects of both small and large concentrations of each element. The element being tested was next added in successive portions to a strongly fluorescent sodium fluoride–uranium bead which was fused, cooled, and examined under the argon bulb for inhibition of uranium fluorescence.

One or more compounds of each element, a mineral containing it, or the free metal were used, and various acid radicals or acid-forming oxides were studied. The elements tested, with the compounds used, were:

Ag (Ag, Ag_2SO_4, $AgNO_3$), Al (kaolin, corundum), As (Realgar, Na_3AsO_4), Au (metal), B ($Na_4B_2O_7$), Ba (barite), Be (beryllonite), Bi (bismutite), Br (KBr), CO_3^{--} (calcite, Na_2CO_3), Ca (calcite), Cb (columbite, Cb_2O_5), Cd (greenockite, $CdCl_2$), Ce [$Ce_2(SO_4)_3$], Cl (NaCl) Co (erythrite, Co_3O_3), Cr (chromite), Cs ($CsNO_3$), Cu (azurite, chalcocite), Er [$Er(NO_3)_3$ crude], Fe (hematite, magnetite), Ga (element), Ge (GeO_2), Hf (cyrtolite), Hg (cinnabar), I (KI), In (metal), K (KF, KBr, KI), La (La_2O_3), Li (Li_2CO_3), Mg (magnesite), Mn (manganite, psilomelane), Mo (molybdenite, $Na_2MoO_4.2H_2O$), Nd [$Nd_2(C_2O_4)_3$], Ni (genthite), PO_4^{---} (Na_2HPO_4, beryllonite), Pb (galena), Rb (RbCl), Re ($KReO_4$), S^{--} (chalcocite, galena), SO_4^{--} (Na_2SO_4), Sb (stibnite), Sc [$Sc(NO_3)_3$], Se (element), SiO_2, etc. (quartz, wollasto-

nite), Sn (cassiterite, SnO_2), Sr (strontianite), Ta (Ta_2O_5, euxenite), Te (metal), Th [$Th(NO_3)_4$], Ti (TiO_2, rutile), Tl [$Tl(NO_3)_3$], V (V_2O_5, carnotite), W ($Na_2WO_4.2H_2O$, wolframite), Y [$Y(NO_3)_3$], Zn (sphalerite, smithsonite), and Zr (zircon, cyrtolite, ZrO_2).

The remaining elements were not tested individually.

Gaseous elements were omitted for obvious reasons. Oxygen as such is without effect, as shown by the fact that neither the oxidizing nor reducing flames influence the fluorescence of uranium in sodium fluoride. The numerous oxides tested failed to show any effect traceable to oxygen in chemical combination.

No preparations containing significant quantities of radium or mesothorium were available.

Hafnium was investigated only to the extent that a positive test for uranium was obtained with cyrtolite from Bedford, N. Y., containing 5.5% of hafnium dioxide (*6*). No unusual fluorescence color or other effect not traceable to the relatively low per cent of uranium in the mineral was noted. Similarly, the rare earths dysprosium, europium, gadolinium, holmium, lutecium, praseodymium, samarium, terbium, thulium, and ytterbium were not examined individually except as they may have been present in the minerals tested. Cerium, lanthanum, and neodymium in the cerium earth group and yttrium and erbium in the yttrium group were found to have exactly similar behavior in the test. A crude ceric nitrate preparation containing most of the rare earths also behaved just like the individual salts. In view of this and their extreme similarity, it did not seem necessary to include the other members. Scandium, however, was tested because of its low atomic weight (45.1) as compared with that of the other rare earths (140 to 175).

INTERFERENCE

A. Production of Fluorescence by Other Elements. Only columbium of all the elements tested caused any fluorescence in the sodium fluoride bead under an argon bulb and Corning No. 9860 filter. In agreement with Papish and Hoag (*10*) it was found that this element when introduced into the bead as columbium pentoxide, or a columbate, causes a faint greenish yellow fluorescence. This was verified by tests made on columbite from Bedford, N. Y., and Colorado, as well as purified columbium pentoxide. Both of the columbites appeared to be free of admixed uranium minerals and neither the Bedford columbite nor the columbium pentoxide darkened a photographic plate in 25 days' exposure. No simple way of distinguishing positively between columbium and uranium fluorescence under all possible conditions was found. The suggestion of Papish and Hoag (*10*) that potassium fluoride instead of sodium fluoride be used is valid, in so far as columbium does not cause fluorescence in potassium fluoride, but is undesirable because of the very much lower sensitivity of the test for uranium in potassium fluoride and the greater inhibiting effect of such elements as iron, manganese, and columbium. It is precisely in looking for a trace of uranium as an impurity in columbium minerals that trouble would be most likely to be encountered.

Any mineral containing uranium as an essential constituent, and almost any containing admixed uranium in excess of about 1% (as discussed below), produces vivid lemon-yellow fluorescence in the sodium fluoride bead under an argon bulb without any filter. This holds true over a considerable range of concentration (which can be approximated by using a sample from the size of a pinhead to half that amount in a bead of about 0.3-cm., 0.125-inch, diameter) providing that the bead is heated until a clear fusion is obtained. On the other hand, columbium, although causing detectable fluorescence at very small concentrations, requires a very much higher concentration than does uranium for the production of fluorescence visible without a filter. Even at the optimum concentration, columbium fluorescence is comparatively faint. As long as the ratio of sample to sodium fluoride is kept within the range suggested above, and no filter is used, columbium will not give a visible fluorescence. This very large intensity difference provides a criterion for distinguishing between the two elements which has been found reliable in the great majority of cases.

However, in testing minerals which do not ordinarily contain uranium for traces of that element, it may be necessary greatly to increase the size of the sample. When the available sample is limited to a few grains of powder, necessitating a reduction in bead size, the ratio of sample to sodium fluoride becomes difficult to estimate. Either factor would tend to vitiate reliance in the concentration-intensity difference given above. This is particularly so if it becomes necessary to use a filter for the detection of very faint fluorescence, as small to moderate concentrations of columbium over a wider range then produce a fluorescence which could readily be confused with that caused by a very low concentration of uranium. In this case the intensity difference cannot be relied upon because certain metallic impurities as discussed below may weaken uranium fluorescence. Nor is the slight color difference reliable, both because columbium fluorescence tends to be more yellowish under the filter, and because various impurities may cause the ordinarily yellow uranium fluorescence to assume a greenish cast. Even so, it will often be possible to distinguish uranium fluorescence with certainty. Thus, a sample of columbite closely associated with euxenite gave a very much stronger fluorescence than the two other columbites referred to above, using like concentrations. However, it would have been impossible to say whether or not a very small trace of uranium was present in the purer columbites.

Examination of such weakly fluorescent beads with a hand spectroscope was tried without success. The two fluorescence spectra are markedly different at high intensity levels, but at very low levels only a narrow green band in either spectrum persists.

It therefore appears that some confirmatory test is required to establish positively the presence of minute traces of uranium, particularly in columbium minerals. A test for radioactivity (interference by thorium) or chemical pretreatment of the sample (see below) suggests itself. In this connection, no direct treatment of the bead was found which would suppress the fluorescence of columbium without affecting that of uranium. Among the expedients tried were:

Use of lithium fluoride in place of sodium fluoride (both uranium and columbium give a pale green fluorescence).

Heating the bead in a blast lamp (fluorescence from both sources is stable).

Addition of potassium chloride to the sodium fluoride–columbium bead (does not completely suppress fluorescence).

Strong (flame) reduction or oxidation of sodium fluoride–columbium bead (only slight effect).

Reduction of sodium fluoride–columbium bead with metallic tin (slight effect).

Addition of titanium dioxide to sodium fluoride–columbium in increasing amounts with strong oxidation or reduction (no effect).

Dissolving sodium fluoride–columbium bead in water, filtering off insoluble matter, evaporation of filtrate, and forming a bead from the residue (fluorescence unimpaired).

The fact that tantalum produces no fluorescence while columbium does provides a simple and moderately sensitive test for the absence of columbium in tantalum preparations.

It is noteworthy that neither the blue fluorescence of neodymium in sodium fluoride nor the green of erbium reported by Nichols and Howes (*8*) was detected with the argon bulb and filter. Neither could fluorescence due to erbium in sodium fluoride be detected with a condensed iron spark (as used by Nichols and Howes). This may have been due to the presence of neodymium (as shown by the absorption spectrum) and no doubt other rare earths, in the crude erbium nitrate used. The above authors found in the course of the same work that many rare earth elements quench the usual cathodo-luminescence of single ones fused into sodium fluoride or other fluxes. A similar

quenching of the fluorescence which should have been caused by erbium may have occurred in the present tests.

On the other hand, a neodymium oxalate preparation when fused into sodium fluoride produced a faint greenish yellow fluorescence and a faint greenish phosphorescence under the iron spark with Corning No. 9860 filter, but not under the argon bulb either with or without the filter. Whether this "off color" luminescence was caused by neodymium or by some impurity in the preparation used is not known. At any rate, no trouble with interfering fluorescence due to rare earth elements is to be expected if an argon bulb is used as the source of ultraviolet. This would seem to be made doubly certain by the probable quenching effect of certain of these elements on the luminescence of others, making it unlikely that the mixtures of rare earth elements occurring in minerals would ever produce fluorescence.

B. Inhibition of Uranium Fluorescence. No instance of specific quenching action was found. In all cases where a dimming of uranium fluorescence was observed, it was caused only by adding other elements far in excess of the amount of uranium and usually approaching the weight of sodium fluoride, or, at least, a large fraction thereof.

Papish and Hoag (*10*) mentioned that certain acidic oxides including silica, titanium dioxide, columbium pentoxide, and others (by inference) are capable of interference. Accordingly, a number of such oxides, or in some cases, the corresponding sodium salts, were tested, with the result that very high concentrations of silica, titanium dioxide, vanadium pentoxide, sodium phosphate, germanium dioxide, and sodium arsenate were found to cause diminution of the intensity of uranium fluorescence. This effect was roughly proportional to the amount added and was most noticeable on cooling the bead immediately after the added substance had fused into it. On slightly longer heating, particularly in the case of sodium arsenate and germanium dioxide, the inhibition was completely removed. A similar removal of the quenching effect was found with the other materials, except that the length of heating required to remove silica was 3 to 5 minutes. The other oxides were increasingly easy to remove, in the order given above. This fact provides a simple means of preventing interference by such materials and one which is superior to the suggestion of Papish and Hoag that additional sodium fluoride be added to dilute silica, or the like below the point at which it can interfere. The latter method has the drawback of also diluting any uranium present in the bead, thus reducing the sensitivity of the test. While such dilution would not be serious in testing a mineral like uranophane, it might easily render the test useless for detecting a trace of uranium in something like fluorescent hyalite.

Similarly, columbium pentoxide and, to a less extent, tantalum pentoxide reduce the intensity of uranium fluorescence when added to the bead in large amounts. However, this effect is limited by the comparatively slight solubility of these oxides in molten sodium fluoride, so that their influence would not ordinarily be troublesome. Attempts to remove these oxides by prolonged heating were unsuccessful, making it necessary to decrease their concentration by adding more sodium fluoride, or by starting over with a larger bead and a smaller sample in case trouble is encountered.

Manganese produces a strong bluish green color when added to the sodium fluoride bead. This coloration, if intense, diminishes or may even suppress uranium fluorescence, possibly in part by rendering the surface of the bead opaque to weak, long-wave ultraviolet. The bead may be decolorized with difficulty by prolonged heating, either in the oxidizing flame, or alternately in a reducing and then an oxidizing flame. The uranium fluorescence thus restored is fainter than it would otherwise have been but would suffice for a test if a filter were used. In general, whenever a bead of any dark color is obtained, it is preferable to start over with a fresh bead and a smaller sample. The bluish green color produced by manganese would suffice for the qualitative detection of that element. It has also been proposed by Hoffmann (*5*) as the basis for a quantitative micromethod. However, it does not seem to offer any particular advantage over the customary sodium carbonate bead test for either purpose.

Borax is unique among the oxy salts tested, in that as the concentration is increased with respect to sodium fluoride, it first reduces the intensity of yellow uranium fluorescence and finally causes inversion to the usual faint green fluorescence of uranium in borax. This fluorescence is visible only with the filter and its spectrum is continuous. It was attributed by Nichols and Slattery (*9*) and by Slattery (*11*) to a uranyl salt.

Of the other acidic oxides and oxy salts tested, sulfate, tungstate, molybdate, perrhenate, and carbonate had no effect whatever, even when added in excess of the sodium fluoride. All halides of the alkali metals were likewise without effect, as were nitrates.

Certain metallic elements were also found to interfere if present in too large excess, although no trouble was encountered when the amounts were comparable to, or less than, that of the uranium. Most prominent of these elements are cerium and the rare earths. Interestingly, scandium, with its much lower atomic weight than the other rare earths, is quite without effect. Interference from an excess of these elements cannot be removed by prolonged heating, nor by strong oxidation or reduction. However, in favorable cases it is possible by long heating to cause rare earth compounds present in excess of their solubility to coalesce and sink to the bottom of the bead, leaving a clear upper portion. When cold, uranium fluorescence will usually be visible with a filter in this clear portion. Also at times, minute droplets of molten sodium fluoride creep up the wire away from the bead during heating. In many cases these droplets will show brilliant uranium fluorescence although the main bead has been completely quenched by the addition of a rare earth compound. This effect depends partly on uranium's being more readily soluble in molten sodium fluoride than the interfering element, and partly on the manner of heating. Neither of these effects is sufficiently reproducible to furnish a reliable means of detecting traces of uranium in the presence of large quantities of rare earths. Interference from these elements is readily avoided by reducing the size of sample as compared with that of the bead, or, if encountered, can be eliminated by adding more sodium fluoride, preferably to a small fragment of the original bead. However, this interference does tend to establish a lower limit of sensitivity to the unmodified bead test as applied to rare earth minerals. At some low percentage of uranium in such minerals it will no longer be possible to keep the concentration of rare earths in the bead below the point of interference without, at the same time, reducing the concentration of uranium below the point at which it could cause fluorescence if present alone in the bead.

A similar interference was observed with magnesium, and to a less extent with calcium, barium, strontium, and aluminum.

Many compounds have a tendency to color the bead if incompletely dissolved, especially if concentrated. In nearly all cases this coloration, except as caused by manganese and cobalt (which latter gives a pale gray blue) can be easily removed by a little longer heating. Since any marked coloration or darkening of the bead is apt to obscure the test, the bead should be heated until clear and transparent when hot, and white or nearly so when cold. Either the oxidizing or reducing flame may be used, depending upon which gives the best results. To illustrate, it was found that wollastonite added to a sodium fluoride–uranium bead produced a grayish nonfluorescent bead when heated at length in a mildly reducing flame (during an attempt to remove the effect of silica). However, fluorescence was completely restored on brief heating again in the oxidizing flame. The gray color and accompanying interference were probably due in part to compounds derived from metallic impurities in the wollastonite. Furthermore, both lead and bismuth when insufficiently heated tend to produce a yellowish color which might conceiv-

ably be mistaken for a faint uranium fluorescence, if examined under an argon bulb without the filter. Other materials tending to color the bead yellowish, or orange to brown, are elemental selenium and indium, sphalerite, many (impure) rare earth salts, and hematite. Readily reducible compounds including those of arsenic, antimony, and bismuth, many sulfides, and silver salts tend to color the bead gray or black unless a strongly oxidizing flame is used (or the sample is previously roasted). Excess uranium, impure rutile, molybdenite, vanadium pentoxide, and thallium nitrate all produced a fugitive brick-red color.

None of the other materials tested caused any interference whatsoever.

APPLICATION OF THE TEST TO URANIUM MINERALS

There seemed to be a need for testing a representative series of uranium minerals in order to establish whether or not the mixtures of other elements present would reduce the reliability of the method. Accordingly, a variety of significant minerals were tested, as shown in Table I. Following the experience gained in checking individual elements, the minerals in Groups B and C were all subjected to prolonged heating in the bead to eliminate the effect of their large content of silica or phosphorus pentoxide.

All the minerals in Group A (containing essential uranium, or uranium in excess of 1 to 2%) gave a positive reaction. Clearly, the other elements in these minerals do not interfere with the test either singly or as mixtures when present in amounts comparable to the uranium, or only exceeding it by small multiples. Specifically, combinations of columbium, tantalum, titanium, rare earths, cerium, thorium, zirconium, hafnium, calcium, copper, vanadium, iron, silica, and phosphorus pentoxide, as represented by the above minerals do not interfere in the least, provided that there is a content of 1, 2, or more % of uranium.

Considering uranophane as typical of the many known pulverulent yellow secondary uranium minerals, it is interesting that other minerals of similar appearance (molybdite, tungstite, bismutite, greenockite) not only do not impart any fluorescence to the bead, but do not interfere seriously with fluorescence due to uranium.

In the case of cerium, rare earth, and/or thorium minerals commonly containing little or no uranium, the inhibiting effect of rare earths becomes much more troublesome. With such minerals the ratio of sample to sodium fluoride is critical, so that it is best to try a very small grain first and then repeat the test with successive small grains. Finally, if the result is still negative, the major portion of the bead should be broken away from the wire and a fresh bead formed over the remaining fragments. By this means one is most likely to find the optimum ratio of sample to sodium fluoride for the particular mineral under test. A filter will generally be required when working in this low range of uranium content. It is also helpful to allow the eyes to become somewhat dark-adapted before examining such a bead for fluorescence as well as to use a blue filter for observing the bead during fusion. This avoids an annoying persistence effect of the sodium flame. In the above tests, for example, a faintly positive test was obtained on the first trial with lovchorrite (15% cerium and rare earths), whereas with cerite (about 60% cerium and cerium earths) the first tiny grain caused a faint fluorescence which was destroyed by the addition of a second grain. This cerite contains about 0.5% U_3O_8 which, to judge from its auto radiograph (*1*), is present about half as specks of an included uranium mineral, and half as uniformly disseminated uranium. The sample appeared free of specks (20× magnification), which would place the limiting concentration of uranium detectable by the direct bead test in a mineral of high rare earth content at 0.2 or 0.3%.

The fluorescence obtained with lovchorrite, cerite, and Arizona gadolinite was definitely yellow, indicating uranium rather than columbium. However, that obtained with the allanite and monazite listed as doubtful was too faint to permit a positive conclusion. Since far less uranium than columbium is needed to cause even this faint fluorescence, thus requiring a smaller chance admixture, and since uranium tends to occur with thorium, it seems probable that the fluorescence was caused by uranium. Based on the result with cerite, it may then be assumed that the minerals listed as doubtful contain somewhat less than 0.2% uranium.

The sensitivity of the test for traces of uranium in minerals containing no interfering elements is well shown by the ease with which a positive test was obtained on hyalite, moss agate, and semiopal. In such cases a much larger sample can be used, providing only that unduly long heating is not required to eliminate the effect of silica. This test, together with the fact that faint green uranyl bands can be detected in the fluorescence spectra with a simple hand spectroscope, affords a nice demonstration that the fluorescence of these minerals is due to included uranyl compounds. The behavior of uranium glass is exactly similar.

Table I. Tests of Uranium Minerals

Mineral	Locality	Approximate % of U_3O_8	Bead Fluorescence[a]
A. Minerals Containing Essential Uranium			
Uraninite	Ruggles Mine, Grafton, N. H.	76 (as U)	++++
Autunite	Bedford, N. Y.	62.7 (as UO_3)	++++
Uranophane	Mitchell County, N. C.	53–67	++++
Torbernite	Avery County, N. C.	55	++++
Carnotite	San Miguel County, Colo.	50–55	++++
Thorogummite	Easton, Pa.	37–43 (25–40% ThO_2)	++++
Ellsworthite	Hybla, Ontario	18–20	++++
Euxenite	Sahamendrevo, Madagascar	15–16	++++
Samarskite	Mitchell County, N. C.	10–13	++++
Thorite	Madagascar	7.7 (65% ThO_2)	+++
Cyrtolite	Bedford, N. Y.	7.3±	+++
Polycrase	Minas Gerais, Brazil	6.5±	+++
Fergusonite	Mitchell County, N. C.	0–7	+++
B. Rare Earth or Thorium Minerals Containing Traces of Uranium			
Lovchorrite	Kola Peninsula	0–0.X	+
Cerite	Jamestown, Colo.	0.5	+
Gadolinite	Kingman, Ariz.	0–0.X	+
Gadolinite	Llano County, Tex.	0–0.X	−
Allanite	Sparta Junction, N. J.	0–0.X	−
Allanite	Yancey County, N. C.	0–0.X	?
Monazite	Yancey County, N. C.	0–0.X	?
Monazite	Idaho	0–0.X	?
C. Miscellaneous Minerals Not Usually Containing Uranium			
Hyalite	Mitchell County, N. C.	?	++
Semiopal	Arizona	?	++
Moss agate	Fremont County, Wyo.	?	++
Columbite	Various	0–0.X	See hdg. Interference, A.

[a] Very strong ++++, strong +++, weak (barely visible without filter) ++, very weak (filter required) +, doubtful ?, absent −.

CHEMICAL PRETREATMENT OF SAMPLE

It was apparent that a simple chemical treatment which would serve to concentrate uranium at the expense of columbium, rare earths, and other interfering elements would be desirable. Several methods were tried, but were abandoned because they were too cumbersome or failed to give a sharp separation. It was eventually suggested (*7*) to the writer that the fact that uranyl nitrate hexahydrate is readily soluble in ethyl ether might be used as the basis of separation. This was found to be the case, and after considerable investigation of the details, the following procedure was adopted:

From 0.3 to 0.5 gram of finely ground sample is mixed with about 10 times as much sodium carbonate and fused in a platinum dish until no further decomposition appears to take place. The cold fusion is dissolved in distilled water plus a slight excess of nitric acid.

The acidified mixture in the original dish is then evaporated to dryness on a steam bath, or controlled hot plate. Care is taken to remove as much acid as possible, in order to avoid an undue amount of iron passing into the subsequent ether extracts. On the other hand, excessive baking tends to lower the amount of uranium which can be extracted.

The residue is then extracted 3 to 5 times with ethyl ether which has been saturated with water. This, plus condensation between extractions, will ensure the presence of enough water to form the ether-soluble hexahydrate of uranyl nitrate. Enough ether is used each time to cover the residue and any lumps are broken up with a glass rod. After a few extractions, the residue will become moist, but not before 40 to 50 cc. of ether have been used, so that this will not interfere with extraction of the major portion of the uranium. The foregoing extraction method was found preferable to the seemingly more efficient liquid-phase extraction of an acid solution of the fusion residue. The latter permitted considerable columbium, possibly as oxyhydrate, to get into the ether extract in colloidal suspension.

Each ether extract is decanted through a dry filter paper into a suitable flask and the combined extracts are evaporated to dryness preferably under a gentle air stream to prevent creeping. At this point macroamounts of uranium will be visible as a deep yellow residue or solution. More or less ferric nitrate and/or whitish insoluble matter may also be present. The latter may consist in part of columbium, tantalum, or rare earth compounds.

The residue from the ether extracts is taken up in 5 to 10 ml. of water and evaporated to dryness. Gentle heating is continued long enough to convert any ferric nitrate to ferric hydroxide. This treatment will also tend to render any traces of columbium, tantalum, or rare earth compounds less dispersible and is therefore essential when seeking a trace of uranium in minerals containing substantial amounts of such substances.

This evaporation residue is taken up in a small volume of water, filtered, and evaporated to dryness. This treatment is repeated until a final residue when taken up in water yields a perfectly clear solution. Two dehydrations will ordinarily suffice, though a third may sometimes be necessary if the amount of uranium expected is extremely small. Four were required in order to obtain a satisfactory blank on a sample of columbium pentoxide and even that was not quite complete. There is no evidence that uranium is lost by dehydration in this treatment, but small mechanical losses will occur unless quantitative washing is used.

To the final solution in a 30-ml. Pyrex beaker about 50 mg. of finely powdered sodium fluoride are added and the solution is again taken to dryness. Heating is continued to dry out the sodium fluoride thoroughly, finally increasing the temperature until decrepitation ceases, while carefully avoiding fusion of the sodium fluoride. The resulting residue should be white or nearly so. It is scraped into a pile and the bead test performed as previously described. A definite yellow fluorescence under the argon bulb without a filter may be taken as conclusive evidence of the presence of uranium in the sample.

Considerably larger samples than that suggested could be handled by making several fusions, extracting each with ether, and combining the extracts.

The method as described is not quantitative, as shown by the fact that a test for uranium was obtained on the sodium nitrate residue from a sample of betafite, even after 5 ether extractions. Complete recovery of uranium could probably be attained by further investigation. However, this is reserved for a later paper.

Assuming that half of the uranium is recovered and considering the ultimate sensitivity of the test, this method has a calculated sensitivity of the order of 0.0001% of uranium in a 0.5-gram sample of such minerals as gadolinite or columbite. Some results are presented in Table II.

Table II. Chemical Treatment of Minerals

Mineral	Locality	Weight of Sample, Gram	Result of Chemical Treatment	Result of Direct Bead Test
Betafite	Madagascar	0.1	++++	++++
Lovchorrite	Kola Peninsula	0.1	++	+
Gadolinite	Kingman, Ariz.	1.23	++++	+
Gadolinite	Llano Co., Tex.	0.28	++	−
Columbite plus trace of uranophane	Bedford, N. Y.	0.55	++++	Entirely inconclusive

SENSITIVITY

The approximate sensitivity of the test was determined by adding a few grains of powdered carnotite to a sodium fluoride bead. This resulted in a fluorescence which was just barely visible without a filter. Since the amount of carnotite added was too small to be seen on the cold bead before fusion, except under 20× magnification, it seems clear that the test is sufficiently sensitive for even the most exacting mineragraphic work.

The sensitivity was next determined quantitatively by adding known amounts of uranium to the bead. This was accomplished by diluting a solution of 2.084 grams of U_3O_8 in nitric acid and adding 0.5 ml. of a solution of various known dilutions to 1.0 gram of finely ground sodium fluoride. Mixing was done in a small porcelain crucible with a glass rod, giving a smooth paste of such consistency that no dry portions remained after mixing and no excess liquid drained out on brief standing. Approximately 50 mg. of this sodium fluoride were taken for each test as determined by weighing the beads after use. An approximately 0.3-cm. (0.125-inch) loop of platinum wire was used. In each test the bead was heated rapidly until clear and perfectly fluid; then was held in that condition for 1 minute.

The results are given in Table III.

Table III. Sensitivity

Test No.	Solution *Mg./ml.*	NaF *Mg./g.*	Approximate Weight of U per Bead *Mg.*	Fluorescence (Unfiltered Argon Bulb)
1	70.69	35.35	1.77	Moderate (a few black specks)
2	2.83	1.4	0.07	Very strong
3	0.113	0.0565	2.8γ	Weak
4	0.0113	5.6γ	0.28γ	Very faint
5	0.0045	2.25γ	0.11γ	Doubtful (without filter) Definite (with filter)
6	0.564γ	0.282γ	0.014γ	None (with filter)

In order to make sure that hydrolysis and precipitation of uranium had not taken place during dilution, another solution corresponding to No. 6 above was made up, adding 6% nitric acid by volume at each stage of the dilution. No fluorescence at all was obtained. A very much more intense source of ultraviolet was found to have no advantage.

The sensitivity may then be expressed as lying between 2.3 and 0.28 microgram of uranium per gram of sodium fluoride, or, based on a 50-mg. bead, as between 0.1 and 0.01 microgram of uranium. This is in fair agreement with the results of Nichols and Slattery (*9*) reported as 1 mole of uranium in 10^7 moles of sodium fluoride (equivalent to 0.6 microgram of uranium per gram of sodium fluoride).

Since the limiting factor is the concentration of uranium in sodium fluoride, the absolute sensitivity in ordinary work where the entire sample is added directly to the bead could be increased by using a smaller bead. Thus, if the mixtures in Table III had been made into 1-mg. beads, the amount of uranium detected would fall between 0.002 and 0.0003 microgram. In this connection Hernegger and Karlik (*3*) found it possible to detect as little as 10^{-10} gram of uranium (0.001 microgram) by using a fluorescence microscope.

Referring again to Table III, the optimum sensitivity using a bead of convenient size and no special equipment is probably of the order of 0.1 to 0.05 microgram of uranium. This may be considered as the working sensitivity for minerals low in rare earths and other nonremovable interfering elements. Such minerals comprise, for example, pitchblende, uraninite, and secondary uranium minerals like carnotite, uranophane, and uraconite.

Nichols and Slattery (*9*) and Slattery (*11*) concluded from spectroscopic evidence that the fluorescence of uranium in sodium fluoride is not due to a uranyl salt.

The former workers obtained a curve showing that as the concentration of uranium is decreased from about 1 mole in 100 of sodium fluoride, the fluorescence intensity first rises until about 2000 moles of sodium fluoride have been added, and then falls off to zero at about 1 mole of uranium in 10^7 or so of sodium fluoride. The shape of this curve plus certain evidence for uranium's being in solid solution led Slattery (*11*) to conclude that she was probably dealing with a Lenard phosphor.

However, it was noted in the course of the present work that when increasing amounts of uranium were added to a sodium fluoride bead, a saturation point was quickly reached beyond which additional uranium simply remained as black specks in the molten bead. Heating for as long as 5 minutes over an ordinary gas burner with maximum air supply failed to dissolve even a very small excess of added U_3O_8. The resulting bead, which had fluorescence of almost maximum brilliance, was preserved as a standard. Another bead was made up in the same way, heating for 5 minutes after each small addition of U_3O_8. After the saturation point had been reached, long heating caused the excess U_3O_8 to be segregated as black specks in the bottom of the bead. The remaining clear portion had, when cold, a fluorescence equal to that of the standard when excess U_3O_8 amounting to about $^1/_{10}$ of the bead had been added. When the excess reached $^1/_3$ of the bead, the clear portion still fluoresced only very slightly less strongly than the standard. In white light it had a very faint pinkish color when cold.

The bead containing $^1/_3$ excess of U_3O_8 was next heated for several minutes over a compressed air–city gas blast lamp, a treatment which dissolved all but a few grains of the excess U_3O_8. The resulting bead was light to dark orange when cold and fluoresced only very faintly. Its fluorescence was stronger in more lightly colored areas, being almost nil in the darkest orange portion.

On the other hand, a bead having maximum fluorescence but containing no excess of U_3O_8 was unchanged by heating in the blast lamp (which also indicates stability to extreme oxidation).

Apparently then, there are two distinct reactions, with the fluorescent complex being formed at a low fusion temperature up to a limit of solubility beyond which any excess uranium remains as black specks of an oxide (or other uranium compound). The latter black material has no effect on fluorescence except to obscure it by encrusting the surface of the bead if present in large enough excess. This excess, on the other hand, is readily converted to an orange compound by heating to a somewhat higher temperature. The small residual fluorescence in lighter colored spots plus the stability of beads containing no excess uranium to this high temperature indicates that any uranium originally present as the fluorescent complex is not changed by higher temperatures, but that this reaction is confined to the excess uranium. The orange compound formed at high temperatures is soluble or dispersable in molten sodium fluoride and is effective in quenching uranium fluorescence. Thus, the resemblance of Nichols and Slattery's (*9*) curve to that of a Lenard phosphor was simply an accident resulting from the fact that they happened to use a fairly high-temperature burner. Had they used a lower flame temperature, the curve would have been nearly horizontal over a wide range of concentrations above 1 mole of uranium in 2000 of sodium fluoride, but would have had the form given for lower concentrations.

In an attempt to identify the orange compound, a 50-gram batch of mixed uraninite and sodium fluoride was heated for 1 hour in a platinum dish over two Meker burners. The mass softened but did not liquefy, and was bright orange when cold. It was extracted repeatedly with hot distilled water until only a brownish crystalline residue was left and the wash water no longer gave a flame test for sodium. After thorough drying, the residue was tested qualitatively and was found to contain sodium, but neither fluorine nor water. It was also infusible, all of which tends to indicate that it was sodium uranate.

For comparison, a strongly fluorescent bead containing only a few black specks of excess uranium was prepared in the low-temperature flame. This bead was dissolved in water and the solution was filtered. Neither the filtrate nor the black residue showed any fluorescence under the argon bulb and No. 9860 filter. On evaporation of the filtrate, a white residue having a faint greenish fluorescence which was unchanged by baking was obtained. Uranyl bands could be seen with a hand spectroscope. This residue when fused on a platinum wire gave a perfectly clear melt which had the expected brilliant yellow fluorescence when cold.

A search of the literature failed to reveal any single uranium compound having all the properties indicated above. Probably the reaction is to be regarded as one in which uranium forms a stable and extremely limited solution in molten sodium fluoride and that this on cooling separates as a distinct fluorescent phase dispersed throughout the sodium fluoride. The ready solubility of the fluorescent complex is against its being anything but a distinct compound. However, its solubility might be explained as resulting from a reaction of water with the uranium whatever its condition, either with or without participation of the sodium fluoride, to form a uranyl compound on subsequent recrystallization of the mixture. The exact nature of the fluorescent phase and the precise mechanism of its formation must be reserved for future investigation.

SUMMARY

1. The fluorescent sodium fluoride bead test for uranium has been found adequately sensitive and reliable when applied directly to any mineral containing uranium as an essential constituent, or in amounts exceeding 1 or 2%. Only an extremely small sample is required.

2. A study of all elements which can reasonably be expected to occur in minerals indicates that only columbium causes a fluorescence which might be mistaken for that of uranium. Suggestions for overcoming this interference based on its greenish color and relatively low intensity are given.

3. In testing for minute traces of uranium in columbium minerals the difference in color and intensity of fluorescence cannot be relied upon. Accordingly, a method of separating traces of uranium from large amounts of columbium by means of ether extraction of uranium as uranyl nitrate hexahydrate was worked out. This makes it possible to detect perhaps as little as 0.0001% of uranium in a 0.5-gram sample of columbite.

4. Further study of the same elements showed that none interfere by reducing the intensity of uranium fluorescence unless present in amounts far exceeding the concentration of uranium needed to produce fluorescence and approaching the weight of sodium fluoride used. No instance of a specific quenching action was found.

5. Of the substances mentioned in 4, interference by silicate, titanates, vanadates, phosphates, germanates, and arsenates, or the corresponding oxides, may be eliminated by prolonged heating of the test bead.

6. Interference by certain metallic elements, notably cerium and the rare earths (except scandium), and to a less extent magnesium, columbium, tantalum, calcium, barium, strontium, and aluminum, cannot be removed by heating. Their interference is overcome by keeping the ratio of sample to sodium fluoride as low as possible. However, this does not entirely suffice when testing for a minute trace of uranium in such rare earth minerals as gadolinite or allanite. The ether extraction method mentioned in 3 can also be used to separate a minute quantity of uranium from large amounts of rare earths.

7. Manganese and certain other elements may cause partial interference by imparting color to the bead. Oxidation, reduction, or longer heating will usually decolorize the bead, though addition of more sodium fluoride may also be required.

8. Chief precaution contributing to the reliability of the test is to heat the bead until a clear fusion is obtained, and to use no more than the smallest required amount of sample. The test bead should be substantially colorless when cold.

9. The sensitivity (roughly 0.05 microgram of uranium) was checked and found to be in good agreement with previous reports. This figure may be considered the practical sensitivity for direct application of the test to uranium minerals low in rare earths and other interfering elements.

10. A preliminary study of the chemistry involved in the test indicated that a solution of uranium in sodium fluoride is formed at any temperature above the fusion point of sodium fluoride, and that this solution on crystallization becomes fluorescent. At low flame temperatures any excess uranium remains undissolved, whereas at higher temperatures the excess is converted to what appears to be sodium uranate which tends to quench the fluorescent phase.

11. The test, coupled with simple examination of the fluorescence spectra, afforded positive evidence that the greenish fluorescence of certain semiopals, moss agates, and hyalites is due to included uranyl compounds.

12. It is concluded that this bead test merits a place among the very few reasonably specific and reliable fluorescence tests known. It compares favorably in these respects with ordinary microchemical tests in general and is vastly superior to the standard ones for uranium.

LITERATURE CITED

(1) Goddard, E. N., and Glass, J. J., *Am. Mineral.*, **25**, 381–404 (1940).
(2) Hernegger, F., *Anz. Akad. Wiss. Wien Math. naturw. Klasse*, No. 2, 15 (1933).
(3) Hernegger, F., and Karlik, B., *Sitzber. Akad. Wiss. Wien Math. naturw. Klasse*, Abt. 2A, 144, 217–23 (1935).
(4) Hoffmann, J., *Sprechsaal*, 73, 153–7 (1940); *Chem. Zentr.*, I, 3963 (1940).
(5) Hoffmann, J., *Sprechsaal*, 74, 155–7 (1941); *Chem. Zentr.*, II, 642 (1941).
(6) Lee, O. I., *Chem. Reviews*, 5, 17–37 (1928).
(7) Lee, O. I., personal communication.
(8) Nichols, E. L., and Howes, H. L., *J. Optical Soc. Am.*, **13**, 573–87 (1926).
(9) Nichols, E. L., and Slattery, M. K., *Ibid.*, **12**, 449–66 (1926).
(10) Papish, J., and Hoag, L. E., *Proc. Natl. Acad. Sci.*, **13**, 726–8 (1927).
(11) Slattery, M. K., *J. Optical Soc. Am.*, **19**, 175–86 (1929).

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NOTES ON ANALYTICAL PROCEDURES

Device for Automatic Protection of a Diffusion Vacuum Pump

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IF A leak occurs in an oil diffusion vacuum system, the hot oil vapor may react with the oxygen of the incoming air to deteriorate the oil and form a sludge which clogs the system. A simple arrangement is herein described which serves to protect the oil in the event of a leak. The scheme consists of utilizing the unbalance voltage developed in a Pirani gage to trigger a thyratron, which in turn opens the diffusion pump heater through a relay.

The complete circuit for alternating current operation is shown in Figure 1. The necessary isolation between the bridge supply potential and the thyratron operating potentials is secured with minimum expenditure for parts through the use of two 5-volt filament transformers. One transformer supplies the tube heaters, and the other transformer—connected in reverse and fed from the 5-volt output of the first—supplies the rectifier anode. Switch S_1 has three positions: center, "off", with the alternate current supply line open; down, "test", with the Pirani galvanometer connected; and up, "stand-by", with the thyratron inserted in place of the galvanometer. The value of the gas pressure at which the pump heater cuts out may be preset by adjustment of the variable bridge arm and the cathode potentiometer.

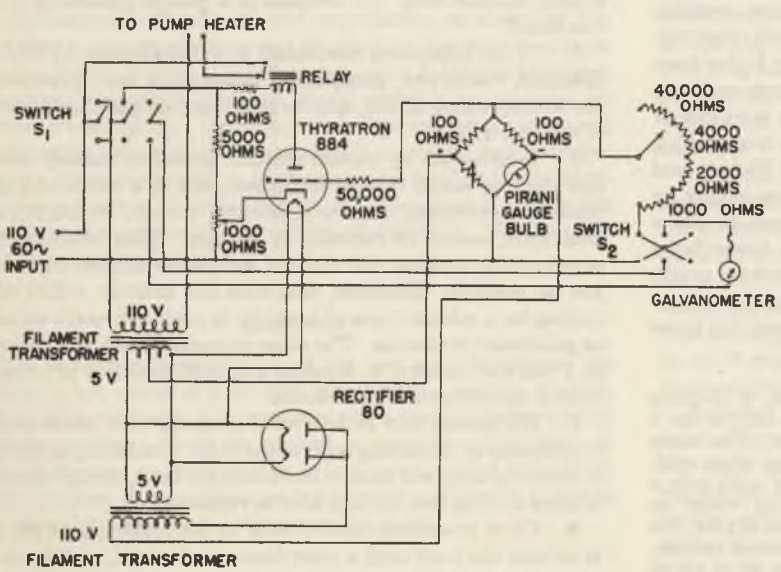

Figure 1. Circuit for Alternating Current Operation

In the particular constructed setup to which the given values of the components in Figure 1 correspond, the indicating meter used in the test circuit was a Triplet 0-100 direct current microammeter, and the relay employed happens to have been a discarded one of unidentified manufacture. The values of the current limiting resistors associated with both the meter and the relay would, of course, probably have to be changed to accommodate different types of equipment in these places.

It is essential that the grid and anode potentials on the thyratron be in phase, or the tube will not fire. To ensure the proper phase relation it may be necessary to reverse the connections to one (any one) of the four transformer windings.

A relay of the lock-in type might be desirable for security. However, in the event of a temporary power interruption a nonlocking relay would serve to restore the pump heat at the appropriate time following the interruption when the pressure is again sufficiently low.

Adjustable Voltage Thermostat System

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AUTOMATIC temperature control is desirable for many laboratory reactions. Thermostats of suitable sensitivity are available, but they usually must be built in the equipment and often utilize several heaters. The assembly described was devised to be used with any one of several standard laboratory electric heaters, to be flexible in the choice of temperature, compact, and low in cost. With any standard single element heater it gives the effect of having a large fixed heater with a small auxiliary control heater.

It was quickly found that regular laboratory heaters used in conjunction with "on-off" thermostats give poor control, because the heater is either completely on or completely off. The circuit in Figure 1 was devised to supply a portion of the heater voltage continuously with an increased voltage upon demand of the thermostat.

A laboratory autotransformer is combined with a thermostat operating a single-pole double-throw switch. (Variacs of 200-, 750-, and 2000-watt sizes—200B, 200CU and CM, and 100Q, respectively, made by the General Radio Corp., Cambridge, Mass.—have been employed for these circuits. They have auxiliary taps 2 and 5 illustrated in Figure 1.)

Approximate voltages supplied, with the transformer wired to supply 0 to 135 volts, are indicated in Figure 2. The circuit is not useful at a setting of less than 15 volts, because of reversal of voltage, as shown in Figure 2. Low voltages are seldom used; for lighter heat loads, lower wattage heaters are usually used and these require a correspondingly higher setting.

If the single-pole double-throw switch were mounted between points 1 and 2 on the input side of the autotransformer, the low voltage would always be approximately 80% of the high voltage. Unfortunately, the switch must then break a very high inductive load and the benefit obtained would not warrant the added cost of the high-capacity switch. In the present assembly, a microswitch (15-ampere capacity) suffices to carry the output load

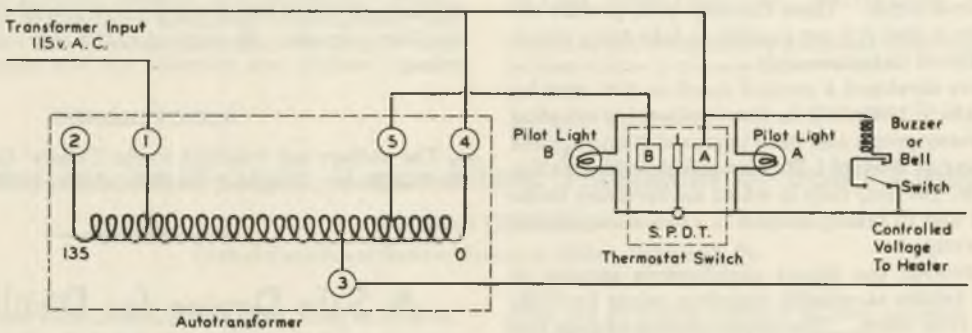

Figure 1. Voltage-Control Circuit

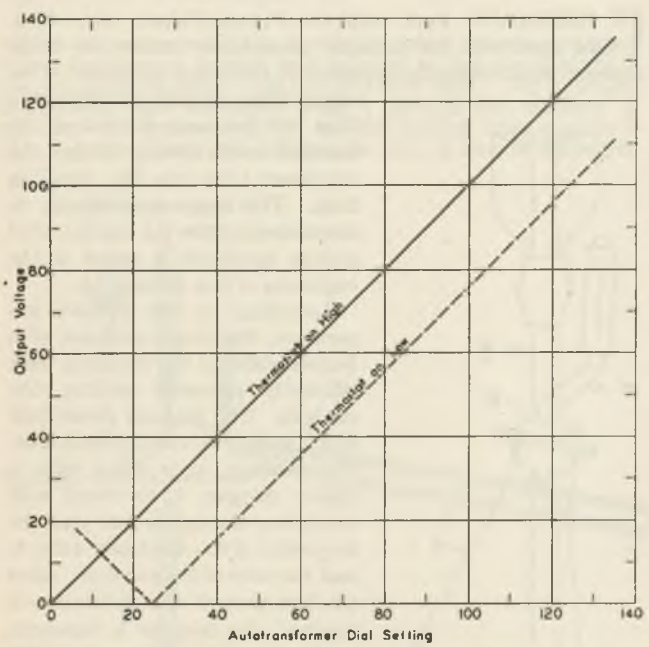

Figure 2. Voltage vs. Dial Setting at High and Low Thermostat Requirements

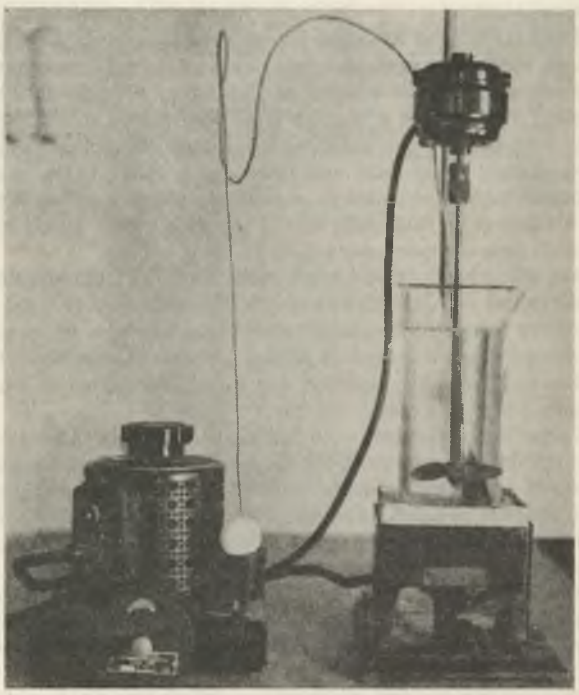

Figure 3. Portable Unit

Combines General Radio Type 200 CM Variac with pilot light *B*

(Microswitch thermostat used was a Type T-2-b Ther-Mu-Trol, Mu Switch Co., Canton, Mass.)

Pilot lights indicated in Figure 1 are 24- or 30-volt bulbs. Operation of the lights is opposite that of the switch—i.e., the light across pole *A* lights when the switch is in contact with pole *B*. Thus the thermostat is on "high" heat when pilot light *B* is illuminated. The addition or substitution of a 24-volt bell or buzzer for pilot light A, with the inclusion of a controlling switch, provides an audible signal that has been found most useful. It permits safe initial heating of a reaction at a voltage higher than that needed to maintain the reaction temperature. When this thermostat setting is attained, the buzzer sounds until the switch is opened. This signal indicates that the Variac setting should be reduced to the proper operating voltage.

Choice of the thermostat depends upon the requirements of temperature control, available space, permissible size of sensitive elements, corrosion problems, etc. The one requirement for this assembly is that it operate a single-pole double-throw switch. Successful operation has been obtained with several types of thermostats.

The apparatus has been used to heat reactions in flasks, beakers, etc. Control at temperatures of 200° to 300° C. is within ±2° C. The principle is applicable to constant-temperature baths, ovens, and other electrically heated laboratory apparatus.

Device for Vacuum Sampling of Liquids and Suspensions

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WHEN sampling liquids and suspensions contained in large tanks, tubes may be inserted at various levels through the tank side or a plunger-operated device may be dipped into the liquid to any desired depth. These methods both present objectionable features in that it is not possible to take many simultaneous samples almost instantaneously.

The authors have developed a method based on that used by McBride in Australia in 1900–1920 for the simultaneous sampling of mine gases at many points and have improved it and applied it to taking as many as a dozen 1-liter samples in sedimentation tanks up to 600 cm. (20 feet) deep in which are revolving thickener paddles, and also to taking samples in viscous suspensions of 1.25 specific gravity.

The method involves the almost simultaneous opening of highly evacuated bottles at suitable sampling points by withdrawing stoppers from them. This avoids the use of taps and makes possible continued operation of the device in abrasive suspensions in which taps could not be expected to remain vacuum-tight.

The conditions of operation preclude the use of anything other than a very easily portable apparatus, and since 1-liter samples are necessary for the authors' application, the whole device is strongly made. The apparatus is of very simple construction, each liter bottle being fitted with a rubber stopper carrying a tube of about 1-cm. bore and 10 cm. long. This has a 6-mm. bore side tube fitted with a piece of rubber pressure tubing closed by a screw clip. The outer end of the wide tube is closed by a greased glass stopper ground to fit inside it.

The whole bottle is held in a wooden cradle by a strong rubber band and as many bottles as may be necessary are clamped by screws or fingernuts and bolts to a ¾-inch iron pipe which may be 25 feet long; if long, it is jointed for ease of assembly. The bottle stoppers are connected one to another by cords which allow a few inches' slack.

In use the bottles are evacuated via the side tube, put each in its appropriate cradle, and the stoppers are connected together. The rod carrying the bottles is introduced to the desired depth into the tank of liquid to be sampled and the stoppers are pulled out by their connected strings. This can be done with only a 1-second interval between the removal of one stopper and the next, and the force necessary to remove each stopper is a good guide to the vacuum in each bottle. Since the bottles fill completely, there is no subsequent contamination of their contents as they are raised to the surface.

The apparatus as described is in use for the weekly investigation of sedimentation rates in tanks ranging from 14 to 20 feet in depth, while a shorter form carrying three bottles is used in tanks about 6 feet deep in which a variety of viscous suspensions up to specific gravity 1.25 is settled.

Various refinements of construction and alteration in the materials from which the device is made should make it applicable to other purposes. In particular, the size could be greatly reduced.

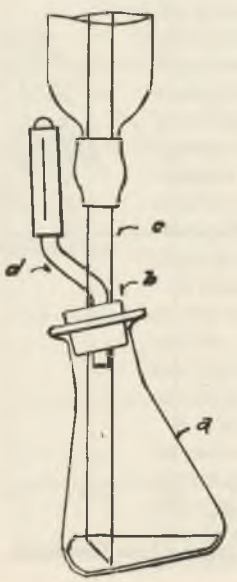
ACKNOWLEDGMENT

The authors are indebted to the Fullers' Earth Union, Ltd., Redhill, Surrey, England, for permission to publish this note.

A Safe Device for Distillation in the Kjeldahl Method

MARIO MENEGHINI, Instituto Biologico, São Paulo, Brazil

IN RESEARCH work with the Parnas-Wagner micro-Kjeldahl apparatus for nitrogen microdetermination in citrus stems, it was frequently necessary to use larger quantities of reagents than usually are employed. This fact increases the risk of the standard acid's flowing through the condenser tube into the distilling flask. This happens principally at the moment when the concentrated sodium hydroxide is added at the beginning of the distillation.



According to the author's experience, the simple addition of a Bunsen valve to the receiving flask efficiently prevents spoiling the analysis. The diagram shows how to adapt the valve to the apparatus. The receiver, *a*, is fitted with a rubber stopper, *b*, furnished with two holes, through which pass the lower end of the condenser tube, *c*, and the tube of the valve, *d*. After the first part of the distillation is finished, the receiver is removed, leaving the rubber stopper with its valve attached to the condenser tube.

Frozen Vitamin Standards

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IN VITAMIN analyses, the preparation of accurate standards is time-consuming and in most laboratories fresh standards are prepared every few days for some vitamins. Glick (*3*) shows the instability of standards and states "that it is unsafe to use a standard solution of thiamine in dilute acid after it has been stored for more than a few days". That riboflavin solutions undergo photolysis was especially shown by the recent work of De Merre and Brown (*2*). The use of frozen vitamin standards is possible both for accuracy and as a time-saving method.

Thiamine hydrochloride and riboflavin standards were prepared in 2% acetic acid containing 10 micrograms per ml. The solutions were made up and further handled in a dark room used for riboflavin studies. Five-milliliter aliquots were pipetted into 15-ml. vials and tightly stoppered with cork stoppers, which had previously been allowed to soak in 2% acetic acid and then in distilled water before drying. The vials were then immediately placed in a refrigerator at about −15° C. and at a 45° angle for freezing and storage. The riboflavin vials were placed in a box to eliminate any light. Ordinary vials will not break on freezing if placed at an angle and if not filled completely.

The frozen standards were tested at various intervals of time and compared with similarly prepared fresh standards. Frozen standards stored for 6 months or for shorter intervals were perfectly satisfactory. The Coleman photofluorometer was used for the measurements and the thiamine was oxidized by the method of Conner and Straub (*1*). The frozen standards were thawed out in a beaker of water at room temperature, which required about 10 minutes. Immediately a 2-ml. aliquot was pipetted out and diluted with 2% acetic acid to give the desired concentration. This was done in the dark room and is important in the case of riboflavin. At least two vials should be thawed each time for checks against improperly stoppered vials and hence moisture loss.

Such frozen standards have proved very efficient, in that a large number of vials can be prepared and used over a long period of time, since the use of the microbalance and the accurate preparation of standards are time-consuming. The method is perhaps also applicable to other vitamins. The use of standard solutions stored at low temperatures is apparently unreliable, particularly in the case of thiamine.

LITERATURE CITED

(1) Conner, R. T., and Straub, G. T., IND. ENG. CHEM., ANAL. ED., **13**, 385 (1941).
(2) De Merre, L. J., and Brown, W. S., *Arch. Biochem.*, 5, 181 (1944).
(3) Glick, David, *Cereal Chem.*, 21, 119 (1944).

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Apparatus for Small-Scale Vapor-Phase Treatment of Solid Compounds

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IN THE vapor-phase dehydrogenation studies being conducted in this laboratory (*1*, *2*) it became desirable to examine the behavior of numerous solid compounds. For this purpose the apparatus shown in Figure 1 was constructed and found satisfactory. The preparation of the catalyst and a portion of the apparatus have been described (*2*).

The solid compound to be treated is packed into the reservoir, *d*, and the mercury leveling bulb lowered, so that the mercury in *e* is just above the level of the nitrogen inlet tube. Dry hydrogen is admitted past the safety trap, *f*, through the capillary, *g*, and into the catalyst bed, *b*. A tube leading from receiver *h* to a bubble counter will indicate the rate of flow of hydrogen.

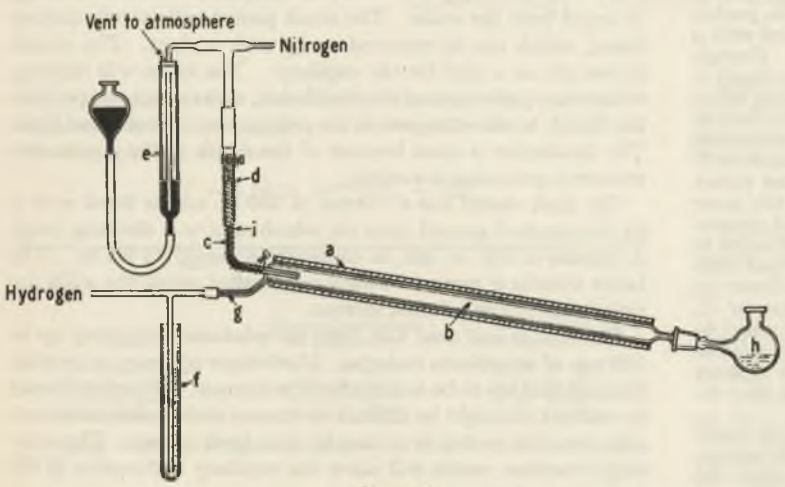

Figure 1

After the hydrogen pressure is once adjusted it should not be changed during the entire experiment.

The catalyst tube is heated to the desired temperature by means of the furnace, *a*, which consists of an iron pipe wound with resistance wire. After the desired temperature is reached, the solid in *d* is melted by the heating coil, *i*, constructed of insulated resistance wire and controlled by a variable transformer. When the compound is liquid, the hydrogen entering through *g* will be observed bubbling up through *d*. A small stream of dry nitrogen is admitted and the mercury in *e* raised until the bubbling through *d* just stops. At this point the pressure on the liquid in *d* is just equal to the pressure in *b*. If the leveling bulb is now raised slightly, the increased pressure causes the melted compound to flow through the 1-mm. capillary, *c*, and into the hot catalyst bed. The head of mercury in *e* controls the rate of addition of the compound.

With a little experience, as little as 1.5 grams of material can be put through the furnace at a fairly uniform rate of about 0.7 gram per hour if desired. Solid compounds of any melting point can be conveniently handled. In smaller furnaces the insertion and removal of catalyst are facilitated by attaching the receiver, *h*, by means of a cork and thus eliminating the constriction in the catalyst tube due to a ⅌ glass joint. Some kind of electrical heater should be placed below *h* to keep the product liquid and prevent clogging.

The author is indebted to John J. Vidosh for the drawing.

LITERATURE CITED

(1) Orchin, *J. Am. Chem. Soc.*, 67, 499 (1945).
(2) Orchin and Woolfolk, *Ibid.*, 67, 122 (1945).

PUBLISHED by permission of the Director, Bureau of Mines, U. S. Department of the Interior.

A Low-Pressure Extraction Apparatus

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IN THE course of work on cottonseed it has become necessary to extract with several solvents at relatively low temperatures and without exposing either the oil or the protein residue to the prolonged heating encountered in the ordinary apparatus of the Soxhlet type. The apparatus evolved in this laboratory is presented diagrammatically in Figure 1 (not drawn to scale).

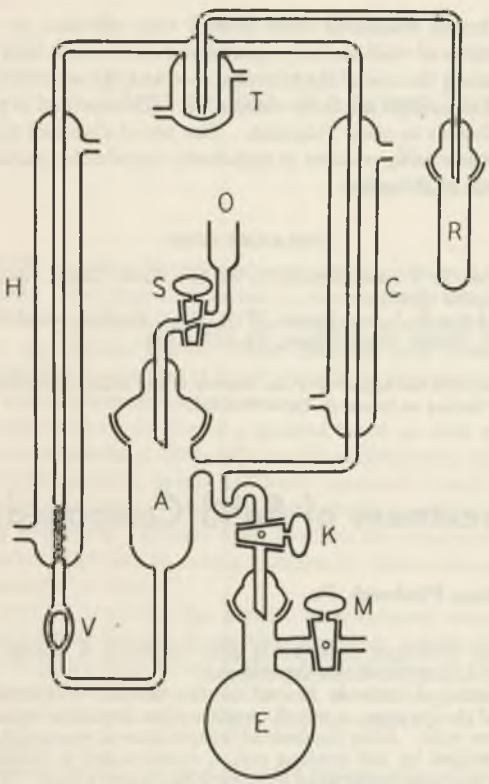

Figure 1. Diagram of Apparatus

The capacity of chamber A is 60 ml.; of chamber T, 20 ml.; of reservoir R, 15 ml.; and of reservoir E, 60 ml. The inner tube of condenser H and the tube leading from chamber T to reservoir R have inner diameters of 3 mm. Condenser H is 40 cm. long.

In the operation of the extraction apparatus, a weighed sample of ground cottonseed is wrapped jelly roll fashion in filter paper, but in such a manner that none of the seed is lost, and the packet is placed in chamber A. The apparatus is then evacuated with a high-vacuum pump, and stopcocks K and M are closed. Enough solvent is added through funnel O to raise the level of the liquid in H to a height of about 3 cm. above the ring seal, care being taken that the vacuum is not lost by the admission of air. Cold water is circulated through condenser C, and warm water is circulated through condenser H and through the outer jacket of chamber T. On the transfer of heat from the warm water in the outer jacket to the liquid in the inner jacket through the walls of the inner tube, the liquid boils with explosive violence. The rapid conversion of the liquid to vapor produces a region of high pressure in the condenser. Flow of the liquid below the ring seal back into chamber A is prevented by valve V, and the vapors are driven up the condenser into chamber T and finally into condenser C, where they are condensed. The condensate is then returned to chamber A. With the equalization of the pressure in the apparatus of the condensation of the vapors in C, liquid from A flows into H because of the hydrostatic head. The process is then repeated.

Regarding the rate of circulation of the solvent, carbon tetrachloride, which has a latent heat of vaporization of 46 calories per gram, circulates at the rate of 3.2 ml. per minute when the temperatures in H and C are 4° and 45° C., respectively, and when the apparatus was evacuated initially to 4×10^{-2} mm. of mercury. With ethanol, which has a latent heat of 204 calories per gram, the rate of circulation is 1.8 ml. per minute.

The extracted oil is deposited on the walls of H as the solvent volatilizes, and is carried up the tube mechanically by the vapors and into chamber T. It is then forced into R by the pressure of the solvent vapor.

Some of the solvent may find its way into R in the normal operation of the apparatus. The solvent may be stripped from the oil by transferring the contents of R to chamber A after the apparatus has been evaluated. The procedure followed is the same as during the extraction, except that there is no seed in A and stopcock K is open so that the condensate from C will flow into E and will not return to A.

Determinations of the oil content of the seed are made by observing the loss in weight of the packet rather than by observing the weight of the extracted oil.

Distillation Flask for Concentration of Solutions in Vacuo

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The Institute of Paper Chemistry, Appleton, Wis.

THE normal round-bottomed distillation flask is often unsatisfactory for concentration of liquids to small volumes. Toward the end of the distillation, the glass capillary often does not touch the liquid or, if it does, it may splatter the liquid all over the flask. Removal of the concentrated solution may be difficult, because of the relatively flat bottom of the flask.

The flask shown one third of actual size in the illustration has been designed for in vacuo concentration of solutions to volumes of 1 cc. or less. The bottom of the flask is cone-shaped to facilitate drainage of liquid from the walls. The small pocket collects the drained liquid, which can be removed easily with a pipet. The pocket serves also as a seat for the capillary. The latter will function to the very last stages of the distillation, and cannot escape from the liquid, as often happens in the ordinary round-bottomed flask. The distillation is quiet because of the depth of the pocket and excessive spattering is avoided.

The flask shown has a volume of 250 cc. and is fitted with a 24/40 standard ground joint for attachment to a distilling head. A volume of 100 cc. can be concentrated easily to 0.5 cc. The latter volume is removed with a pipet, after which the walls are rinsed with 0.5 cc. of fresh solvent.

The author has used this flask for solutions containing up to 100 mg. of amorphous material. If solutions of sirupy or crystalline material are to be concentrated to dryness, the pocket should be omitted; it might be difficult to remove such a solute mechanically from the pocket or to dissolve it in fresh solvent. The cone-shaped bottom would still allow the capillary to function in the last stages of the concentration.

A Nomograph for Emergent Stem Correction of Thermometers

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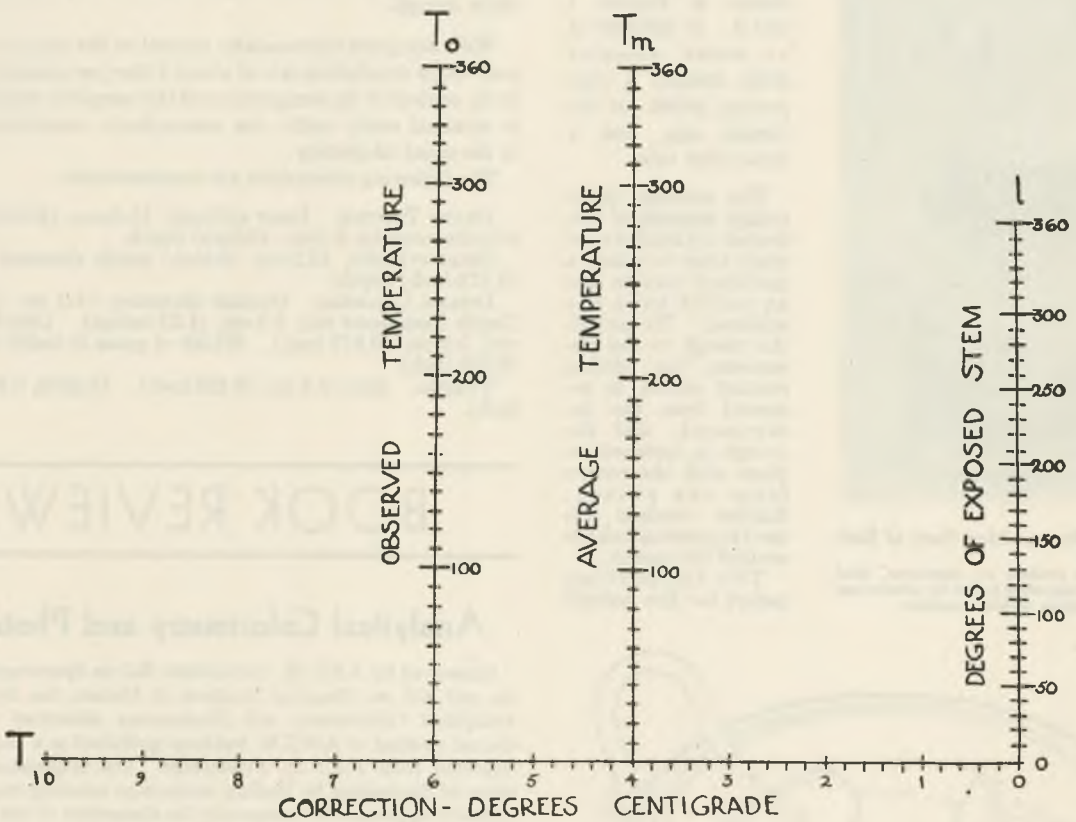
IT IS frequently necessary to apply correction factors to thermometers calibrated for total immersion but used at partial immersion. The values given in various references are somewhat different and involve the use of a formula (*1–4*).

A survey of domestic thermometer manufacturers indicates that, in general, the bulbs of ordinary range (0° to 360°) thermometers are constructed of Corning Normal glass similar to Jena 16 III, while the stems are made of a soda-lead glass known as Corning G4D. (Corning G4C glass has been replaced by G4D.) Thermometers for higher ranges are normally constructed of a domestic borosilicate glass similar to Jena 59 III. The linear coefficient of expansion of these glasses and the apparent cubical expansion of mercury in these glasses are given in Table I.

where

T = correction in ° C.
1.56×10^{-4} = apparent coefficient of expansion of mercury in glass
l = length of exposed stem in ° C.
T_o = observed temperature
T_m = average temperature of exposed stem

To use the nomograph, a line is drawn through T_o and T_m across the nomograph. Using a 90° angle, such as a triangle or the corner of a sheet of paper, a line is drawn at right angles to the above line through the proper point on scale l. Where this line intersects the T axis determines the correction. A transparent sheet containing two lines crossing at 90° will be helpful to frequent users of the nomograph.



The corrections to be added to the observed temperature are obtained from the nomograph, in the construction of which the following equation was used:

$$T = 1.56 \times 10^{-4}\, l(T_o - T_m)$$

Table I. Expansion

Glass	Linear Coefficient of Expansion	Calculated Apparent Cubical Expansion of Mercury in Glass[a]
Corning Normal	8.8×10^{-6}	1.55×10^{-4}
Corning G4C	8.6×10^{-6}	1.56×10^{-4}
Corning G4D	8.4×10^{-6}	1.57×10^{-4}
Borosilicate	6.0×10^{-6}	1.64×10^{-4}

[a] Cubical coefficient of expansion of mercury taken as 0.18186×10^{-3} at 20° C.

When the correction is greater than 5°, it is advisable to add the correction to T_o and recompute a new correction. For high-temperature borosilicate thermometers the correction determined from the nomograph should be multiplied by 1.06.

ACKNOWLEDGMENT

The assistance of T. C. Patton of the Baker Castor Oil Company in constructing the nomograph is appreciated.

LITERATURE CITED

(1) Adams and Johnson, "Laboratory Experiments in Organic Chemistry", 3rd ed., p. 35, New York, Macmillan Co., 1940.
(2) Berl and Kullman, *Ber.*, 60, 815 (1927), a similar, though not identical, nomograph.
(3) Busse, "Temperature, Its Measurement and Control", p. 237, New York, Reinhold Publishing Corp., 1941.
(4) Reilly and Rae, "Physico-Chemical Methods", 3rd ed., Vol. 1, p. 370, New York, D. Van Nostrand Co., 1939.

Temperature-Control Device for MacMichael Viscometer

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THE conventional MacMichael viscometer is equipped with an adjustable heater which lacks positive thermostatic control. The equipment described here was devised to maintain the sample at constant temperature by immersion of the sample cup in a bath through which water is circulated continuously from a thermostatically controlled reservoir.

Figure 1. Disassembled Parts of Bath

Drain trough in position on viscometer, inlet gooseneck and supporting jacket for sample cup raised above working positions

The bath assembly, which can be attached to the viscometer without permanent modification thereof, is shown in Figures 1 and 2. It consists of an outer, annular drain trough, a supporting jacket for the sample cup, and a water inlet tube.

The annular drain trough consists of two brazed cylinders of sheet brass to which a machined bottom and an outlet tube are soldered. To attach the trough to the viscometer, the sliding contact switch is removed from the instrument, and the trough is fastened in place with the screws from the switch. Rubber washers are used to prevent leakage around the screws.

The supporting jacket for the sample cup, together with its integral channel for intake of water, is turned from solid brass stock. The body of the jacket has the same outside dimensions as the outer cup supplied with the instrument. The baffles, drive studs, and outlet tube are soldered in place. A bead of solder on the outer rim facilitates measurement of the rate of rotation.

Water at constant temperature is introduced into the intake channel of the jacket through a small gooseneck. The gooseneck is clipped to the outer edge of the drain trough, as shown in Figure 2, and is inclined about 60° in the direction of rotation of the jacket to minimize the development of a standing wave in the intake channel.

The path of the water through the bath assembly is shown by the arrows in Figure 2. Water from the intake channel flows through gate *A* into the space between the sample cup and its supporting jacket, where it is deflected downward and across the bottom of the sample cup by the two vertical fins, *F*, on the inside of the jacket. The overflow empties through gate *B*, which is isolated from the intake channel by dams *D*, and spills into the drain trough.

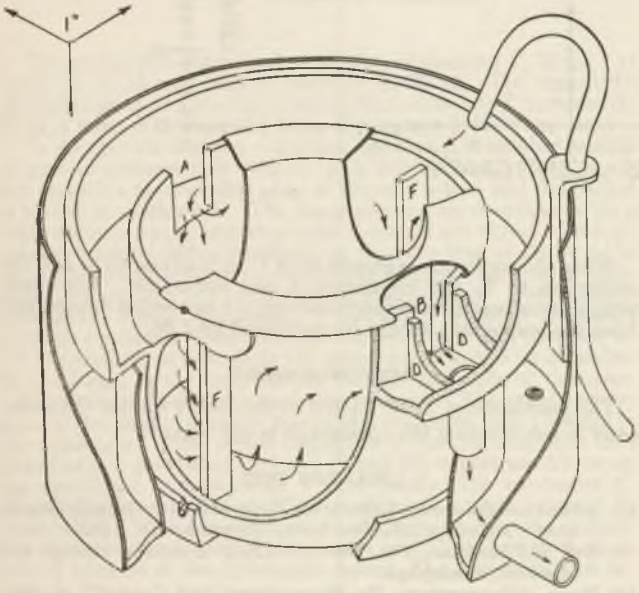

Figure 2. General Construction of Bath

Sample cup in position with its supporting jacket fitted into turntable of viscometer. Arrows indicate flow of water

With adequate thermostatic control of the source of the water and with a circulation rate of about 1 liter per minute through the bath, control of the temperature of the sample to within ±0.1° C. is attained easily under the atmospheric conditions prevailing in the usual laboratory.

The following dimensions are recommended:

DRAIN TROUGH. Inner cylinder, 11.0-cm. (4.3125-inch) outside diameter by 3.1-cm. (2-inch) depth.

Outer cylinder, 15.2-cm. (6-inch) inside diameter by 8.6-cm. (3.375-inch) depth.

INTAKE CHANNEL. Outside diameter, 14.0 cm. (5.5 inches). Depth from outer rim, 3.2 cm. (1.25 inches). Depth from inner rim, 2.2 cm. (0.875 inch). Width of gates in inside rim, 1.6 cm. (0.625 inch).

TUBING. Inlet, 0.5 cm. (0.188 inch). Outlets, 0.8 cm. (0.3125 inch).

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