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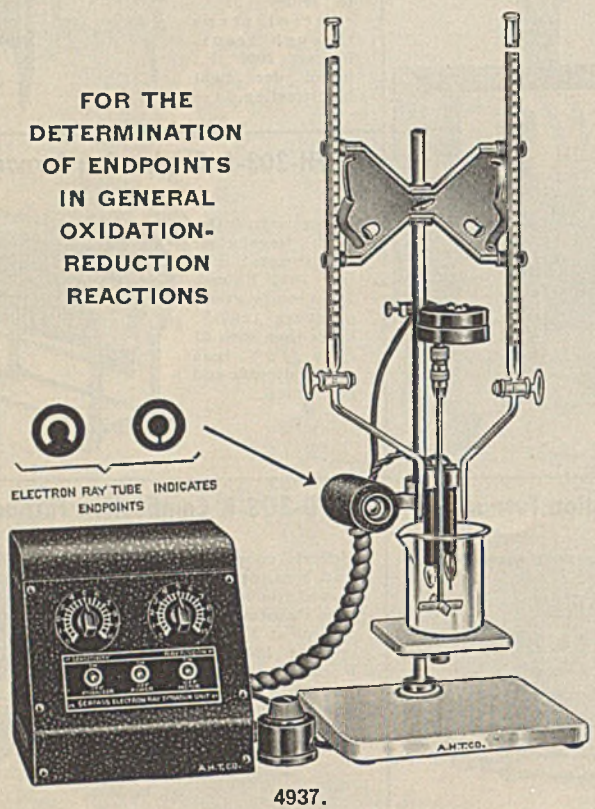

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INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

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Quantitative Spectral Analysis of Fats

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THE Kaufmann (*6*) method of analysis for oleic, linoleic, and linolenic acids in some fats is based on determinations of the iodine number, thiocyanogen number, and saturated acid content of the mixed fatty acids. The analysis for the saturated acids alone involves an iodine number determination of the separated "solid" acids and, depending upon the method used, may also involve a thiocyanogen number determination in order to correct for the unsaturated acids which are not completely separated from the solid acids.

Until recently, analyses of fats made by the Kaufmann method were in error, because theoretical values were used for the thiocyanogen numbers of linoleic and linolenic acids. Waterman *et al.* (*15*), Riemenschneider and Wheeler (*12*), and Kass *et al.* (*3*, *4*), however, found the values for linoleic and linolenic acids to be empirical. Although thiocyanogen numbers are reliable when determined under carefully controlled conditions, it is desirable to have a more direct method for analysis of the linoleic and linolenic acid content of fats.

Prolonged heating of fats during saponification has been observed by Moore (*9*) to cause nonconjugated double bonds in fatty acids to become conjugated and thereby to absorb radiation in the ultraviolet region. Kass *et al.* (*5*) and Miller and Burr (*7*) reported that it is possible to measure quantitatively, by spectral absorption, the amount of linoleic acid in vegetable oils after heating them with a 1 to 4 solution of potassium hydroxide in ethylene glycol. The acids affected by this treatment are the unsaturated ones containing more than one double bond. In many fats the unsaturated acids consist chiefly of oleic, linoleic, and linolenic acids.

Isomerization by alkali has been studied and made the basis for the method of analysis described in this paper.

Experimental Procedure

The absorption data were obtained from measurements made with a photoelectric spectrophotometer (*2*, *8*), employing a Hilger double monochromator with crystal quartz optics. As a source of ultraviolet radiation, a Munch (*10*) hydrogen discharge tube with a fused quartz window was used. In the discussion of the results, the term "specific alpha" will be used:

$$\text{Specific } \alpha = \frac{\log_{10} \frac{I_0}{I}}{cl}$$

where α = absorption coefficient
I_0 = intensity of radiation transmitted by the solvent
I = intensity of radiation transmitted by the solution
c = concentration of solute in grams per 1000 ml.
l = length in centimeters of solution through which the radiation passes

The first procedure tried in carrying out the isomerization was to reflux a weighed sample of fat in a 1 to 4 solution of potassium hydroxide in ethylene glycol for 25 minutes, then free the fatty acids with hydrochloric acid and extract them with ether.

Consistent results were not obtained by this method, and difficulty was experienced with emulsions in some cases. During heating of ethylene glycol and potassium hydroxide alone as a blank a substance which absorbs ultraviolet radiation is dissolved from the glass vessel or is formed from the reagents, and is extracted during the ether extraction. This, together with the fact that some oxidation of the easily oxidized conjugated double bonds may occur during the manipulation, probably accounts in part for the inability to obtain consistent results by this procedure.

Temperature is important in the rate of isomerization. Another difficulty was encountered because the boiling point of ethylene glycol–potassium hydroxide solution varies with the amount of water derived from the potassium hydroxide. The analytical reagent grade of this alkali contains about 10 per cent water. The temperature of reflux varies with the type of condenser used.

To overcome the latter difficulty, samples were heated at a temperature below the boiling point. It was found that a much weaker solution of alkaline glycol than the 1 to 4 solution previously used would serve for the isomerization. A concentration of 7.5 grams of potassium hydroxide, assaying 85 per cent potassium hydroxide, per 100 ml. of ethylene glycol, was adopted. This solution was 1.3 *N* with respect to potassium hydroxide. In preparation of the reagent, the alkaline glycol solution was boiled in an Erlenmeyer flask until the temperature reached 190° C. This removed most of the water, and thus it was possible to maintain a constant temperature of 180° C. while heating the samples.

The oil bath for heating the samples was maintained at 180 ± 0.1° C.

It was possible to simplify the method and to obtain consistent results by making absorption measurements directly on the soap solution, using a blank solution in the solvent cell. Thus the troublesome procedure of freeing the fatty acids and extracting them was found unnecessary.

A high dilution of the original reaction mixture is required before spectroscopic readings are taken. Ethanol is very suitable for this purpose, since the soaps are completely soluble in this solvent and do not foam when the solution is shaken. Ethanol is easily purified for optical use by distillation over potassium hydroxide and zinc dust.

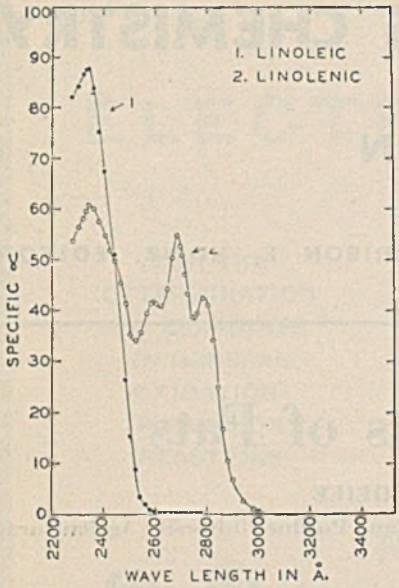

FIGURE 1. ABSORPTION SPECTRA OF POTASSIUM LINOLEATE AND POTASSIUM LINOLENATE FORMED BY ALKALI TREATMENT OF ETHYL ESTERS

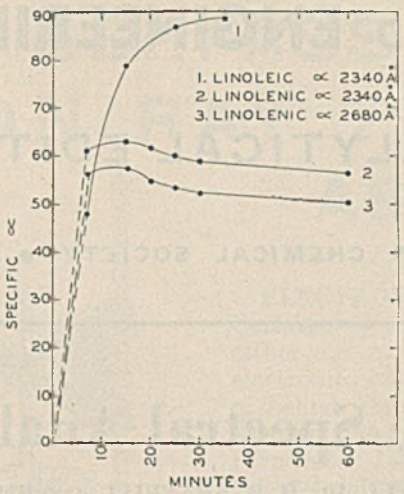

FIGURE 2. EFFECT OF TIME OF HEATING AT 180° WITH ALKALI ON SPECIFIC ABSORPTION COEFFICIENTS OF LINOLEIC AND LINOLENIC ACIDS

Only one band is present in the isomerized linoleic acid and it is due to diene conjugation. The specific alpha value at 2340 Å. is 87.1. The band with a maximum of 53.7 at 2680 Å. is due to triene conjugation, while the maximum of 60.0 at 2340 Å. in this same curve is due to diene conjugation in the isomerized linolenic acid. The reproducibility of these values is illustrated in Table I. The determinations were made at several different times and with alkaline glycol which was made up fresh each time.

In analysis, the intensity of absorption at 2680 Å. is a measure of the amount of linolenic acid present, while the intensity of absorption at 2340 Å. is a measure of both linoleic and linolenic acids. In case both acids are present, a correction is made for the absorption at 2340 Å. which is due to diene conjugation resulting from the linolenic acid.

Method

Weigh out accurately about 0.1 gram of fat or fatty acids into a small vial of the type used for iodine number determinations. Add 10 ml. of the alkaline glycol reagent, with a pipet, to a 15 × 2.5 cm. (6 inch × 1 inch) test tube and place in an oil bath at 180° C. Cover the tube with a loosely fitting glass top. The tubes should always be immersed in the bath to a constant depth. When the temperature of the reagent in the test tube has reached 180° C., drop in the vial containing the fat sample. Swirl the tube three times at 1-minute intervals to mix the fat with the glycol solution.

At the end of 25 minutes remove the tube and cool rapidly under the tap. Transfer the contents of the tube quantitatively to a 250-ml. volumetric flask, using ethanol to wash out the tube, and dilute to volume with 99 per cent ethanol.

Allow the samples to stand in a refrigerator for 5 or 6 hours, or overnight. At the end of this time, material removed from the glass by the hot alkaline solution will have precipitated. Bring the solution in the volumetric flask to room temperature and filter a portion of the solution. Make proper dilutions for absorption measurements using 99 per cent ethanol.

It is necessary to carry a blank solution, consisting of alkaline glycol, throughout the whole of the procedure, including dilutions, for use in the solvent cell.

Preparation of Standards

Linoleic and linolenic acids were prepared by bromination (*13*, *14*) and debromination (*11*) of the mixed acids of corn oil and linseed oil, respectively.

The tetrabromide, after several recrystallizations from petroleum ether, had a melting point, obtained with a total immersion thermometer, of 115.5° C. The ethyl linoleate prepared by debromination of this product with zinc and 7.5 *N* anhydrous ethanolic hydrochloric acid had an iodine number of 163.8; theory 164.4.

The hexabromide, after several recrystallizations from xylene, had a melting point, obtained with a total immersion thermometer, of 185.5° C. The ethyl linolenate obtained by debromination with zinc and 7.5 *N* anhydrous ethanolic hydrochloric acid had an iodine number of 247.1; theory 248.8.

In obtaining standard absorption values for these acids, the esters were weighed and a conversion was made by a factor to obtain the equivalent weight of acid. Samples of approximately 0.1 gram were used and treated as outlined previously.

Results and Discussions

Figure 1 shows the absorption curves obtained for the potassium soaps of linoleic and linolenic acids after the alkali isomerization.

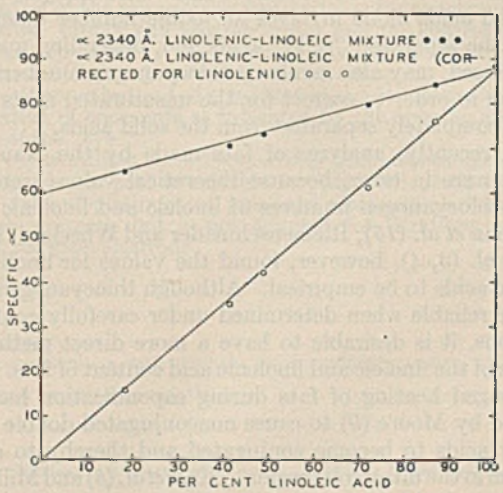

FIGURE 3. RELATIONSHIP OF SPECIFIC ALPHA VALUES AT 2340 Å. AFTER ISOMERIZATION TO PER CENT LINOLEIC ACID IN LINOLEIC-LINOLENIC ACID MIXTURE

The effect of time on the course of the reaction was studied with both linoleic and linolenic acids. The absorption rises rapidly at first (Figure 2), indicating rapid conversion of the double bonds to conjugated positions. The maximum absorption at 2340 Å and 2680 Å. is reached at the end of about 15 minutes with linolenic acid and then begins to drop gradually. The absorption of linoleic acid continues to rise slowly with

TABLE I. STANDARD VALUES FOR USE IN ANALYSIS

For Linoleic Acid (Isomerized), Specific α at 2340 Å.	For Linolenic Acid (Isomerized) Specific α at 2340 Å.	Specific α at 2680 Å.
87.1	60.1	53.7
86.9	60.1	53.6
87.2	60.1	53.6
87.1	59.7	54.0
Av. 87.1	60.0	53.7

TABLE II. ANALYSES OF LINOLEIC-LINOLENIC MIXTURES BY SPECTROSCOPIC METHOD

Observed Alpha, 2680 Å.	Linolenic Found	Linolenic Added	Difference in Composition	Error	Alpha 2340 Å.[a]	Linoleic Found	Linoleic Added	Difference in Composition	Error
L./g. cm.	%	%	%	%	*L./g. cm.*	%	%	%	%
7.42	13.8	13.4	+0.4	+2.99	76.1	87.4	86.6	+0.8	+0.92
14.8	27.6	28.0	−0.4	−1.43	62.2	71.4	72.0	−0.6	−0.83
27.4	51.0	51.1	−0.1	−0.20	42.2	48.4	48.9	−0.5	−1.02
31.8	59.2	58.3	+0.9	+1.54	35.0	40.2	41.7	−1.5	−3.60
44.0	81.9	81.5	+0.4	+0.49	15.7	18.0	18.5	−0.5	−2.78
			Av. ±1.33					Av. ±1.83	

[a] Corrected for the absorption of linolenic acid at 2340 Å.

TABLE III. COMPARISON OF ANALYSES BY SPECTROSCOPIC AND KAUFMANN METHODS

Sample	Iodine No. (Glyceride) Wijs Method	Linolenic Acid S	Linolenic Acid K	Linoleic Acid S	Linoleic Acid K	Oleic Acid S[a]	Oleic Acid K	Saturated Acids S[b]	Saturated Acids K
		%	%	%	%	%	%	%	%
Linseed	179.7	47.3	46.8	18.2	22.4	28.6	22.8	5.9	8.5
Soybean 1	136.5	9.88	8.95	53.2	53.7	21.6	23.3	15.4	14.0
Soybean 2	134.3	7.47	8.03	54.7	53.5	23.4	23.9	14.5	14.5
Soybean 3	130.0	5.23	6.23	56.2	52.0	21.8	27.3	16.8	14.5
Cottonseed	110.1	0.00	0.00	54.8	54.2	17.8	18.9	27.4	26.9

[a] % oleic acid = $\dfrac{\text{I No. of mixed acids} - (\%\text{ linolenic})(\text{I No. of linolenic}) - (\%\text{ linoleic}) \times (\text{I No. of linoleic})}{\text{I No. of oleic}}$.

[b] % saturated acids = 100% − % unsaturated acids.

time. The change is not critical at the end of 25 minutes, and this period was used in the method of analysis.

Known mixtures of linoleic and linolenic acids were made up and analyzed by the spectroscopic method. The alpha values at 2340 Å. fall on a straight line (Figure 3) when plotted against the percentage of linoleic acid. The top line represents the total alpha values at 2340 Å. due to both linoleic and linolenic acids together, while the lower line represents the alpha values after correction for the linolenic acid which was determined by the absorption band at 2680 Å.

The alpha values at 2680 Å. for various mixtures of linoleic and linolenic acids have been plotted (Figure 4) against the percentage of linolenic acid. These values fall on the same straight line with those obtained for a mixture of stearic and linolenic acids. It is obvious that neither the saturated acids nor linoleic acid interfere with the determination of linolenic acid.

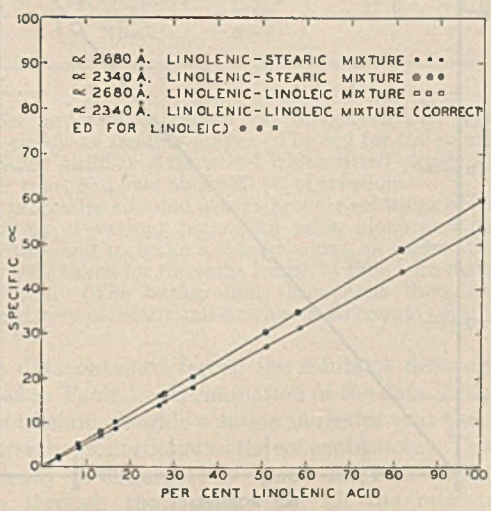

FIGURE 4. RELATIONSHIP OF SPECIFIC ALPHA AT 2340 Å. AND 2680 Å. TO PER CENT LINOLENIC ACID IN LINOLENIC-LINOLEIC AND LINOLENIC-STEARIC MIXTURES

The alpha values at 2340 Å., after correction for the absorption due to linoleic acid, have been plotted and fall on the same straight line with those obtained for a mixture of stearic and linolenic acids.

The actual percentages of linoleic and linolenic acids which were added to the mixtures and the percentages which were determined by the spectroscopic method are compared in Table II. Linolenic acid was determined with a percentage error of ±1.33 and linoleic acid was determined with a percentage error of ±1.83.

For the analysis of a fat (which consists chiefly of saturated acids, oleic, linoleic, and linolenic acids) by the spectroscopic method it is necessary to know only the iodine number of the oil before isomerization in addition to the amount of linoleic and linolenic acids in order to calculate the percentage of oleic acid. The saturated acids are then obtained by difference. Several samples of soybean oil, linseed oil, and cottonseed oil were analyzed by both the Kaufmann and the spectroscopic methods. The results are compared in Table III.

The saturated acids in the Kaufmann method were determined by crystallization from acetone at −40° C. (*1*).

Conclusions

A spectroscopic method is described for direct determination of the linoleic and linolenic acid content of a fat. These acids can be determined very simply and as accurately as standard values for the pure acids can be obtained, when the fats do not contain other acids with two or more double bonds. Making use of the iodine number, the oleic acid content can be obtained; the saturated acids are then obtained by difference. Thus an analysis can be obtained on many fats (those containing chiefly saturated acids, oleic, linoleic, and linolenic acids) with as little as 0.2 gram of sample.

The method is more rapid than the Kaufmann method and involves fewer determinations.

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Analysis for Potassium by Its Natural Radioactivity

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PRESENT-day knowledge of nuclear physics suggests that radioactive isotopes of all the elements probably existed when the earth was formed. However, only members of the uranium-radium, uranium-actinium, and thorium series and the elements potassium, rubidium, and samarium still show appreciable natural radioactivity. (There is also some evidence that lutecium and neodymium have weak activity, *8*.) The survival of these is explained by the fact that they have half-lives of the order of 10^9 years, which is so long that they exist yet in detectable quantities. All the rest of the radio-isotopes originally present must have had comparatively short life periods and consequently have completely decayed during the ages, or else they decay so slowly that they cannot be detected readily. Evidence of this is the fact that the three hundred or more radio-isotopes already produced artificially have half-life periods ranging from a few seconds to only a few years. Only two, B^{10} and C^{14}, have half-lives greater than 10^3 years, and even such periods seem short in comparison with geologic time.

One of the pieces of evidence which proved that potassium was naturally radioactive was the observation that the activity of a given sample is directly proportional to its potassium content (*6*). Apparently, however, no attempt was made to apply this to the quantitative analysis of materials for their potassium content. [Since this work was completed, Fenn, Bale, and Mullins (*5*) have described determinations of the radioactivity of potassium from bone ash with a dipping counter tube. However, these authors did not attempt to develop a procedure for routine potassium analyses.] Such an application, were it possible to carry out simply, would appear to be of great value, since the determination of potassium in the presence of other alkalies and certain anions is a difficult and time-consuming procedure. Accordingly, the work recorded here was undertaken in order to establish a rapid method of analysis for potassium, based on its natural radioactivity, which would be free from interference by other elements and would lend itself to routine operation. The experiments described here show the practical nature of this method, and indicate as well the various factors affecting such a determination.

Three of the questions which bear on the subject may be briefly disposed of here. (1) One concerns the possible interference by the other natural radio-elements. Of these rubidium causes no concern, for it is very rare in occurrence and emits a soft beta-ray, only one third as energetic as that of potassium, and no gamma-ray. Samarium too is very rare, and furthermore, since it is an alpha-emitter, it would not be counted in the tube employed. However, the members of the uranium or of the thorium series would, if present, invalidate the potassium analysis by their radioactivity. Since these elements are met only rarely, they do not seriously restrict the application of the method, and, even if present, they can be removed comparatively easily by standard procedures.

(2) A second factor has to do with the natural rate of decay of the potassium. The well-known law for radioactive disintegration is

$$N = N_0 e^{-\lambda t}$$

where N is the number of radioactive atoms at time t, N_0 is the number initially present, and λ is the decay constant. For potassium $\lambda = 1.56 \times 10^{-17}$ sec.$^{-1}$ which in terms of half-life is 1.4×10^9 years (*1*). Consequently, no detectable change would occur in activity of the potassium in the time required for analysis.

(3) The naturally occurring activity of potassium is due to the isotope of mass number 40 (*13*). Isotopes 39 and 41 are stable, and the abundance of masses 39, 40, and 41 are 93.3, 0.012, and 6.7 per cent, respectively (*2*, *9*). The analytical method developed here depends, consequently, on the assumption that no isotopic separation occurred during any of the ordinary processes to which the sample may have been subjected. This assumption seems reasonable, for the appreciable separation of isotopes of such high atomic weight by the usual chemical and physical procedures is negligible. Moreover, the constancy of the ratio of the potassium isotopes in mineral matter has been fairly well established. Smythe (*12*) and Brewer (*3*) have shown that there is no variation either in the K^{39}/K^{43} or in the K^{39}/K^{41} ratio in various rocks, in sea water, and in commercial potassium. A slight separation seems to be discernable in plant and animal specimens, but the amount and the direction of the isotopic shift are variable (*4*, *5*). Such small changes would be completely unimportant for the purposes at hand.

Experimental

The apparatus used for the preliminary experimental work consisted of a small Geiger counter tube in a lead shield, an amplifying and recording circuit, and a glass cell for the solutions. The Geiger tube was of thin-walled glass about 1.5 cm. in outside diameter and 15 cm. long, silvered on the inside wall. It was filled to about 100-mm. pressure with a "fast" counting gas consisting of 90 per cent argon and 10 per cent alcohol. The

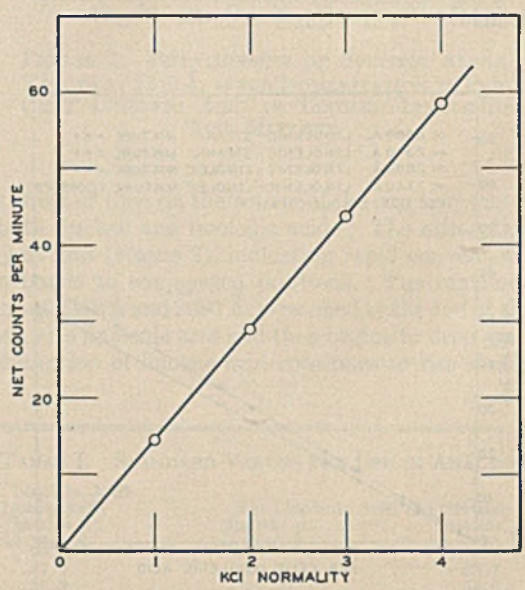

FIGURE 1. COUNTING RATE *vs.* POTASSIUM CONCENTRATION
Small counter tube

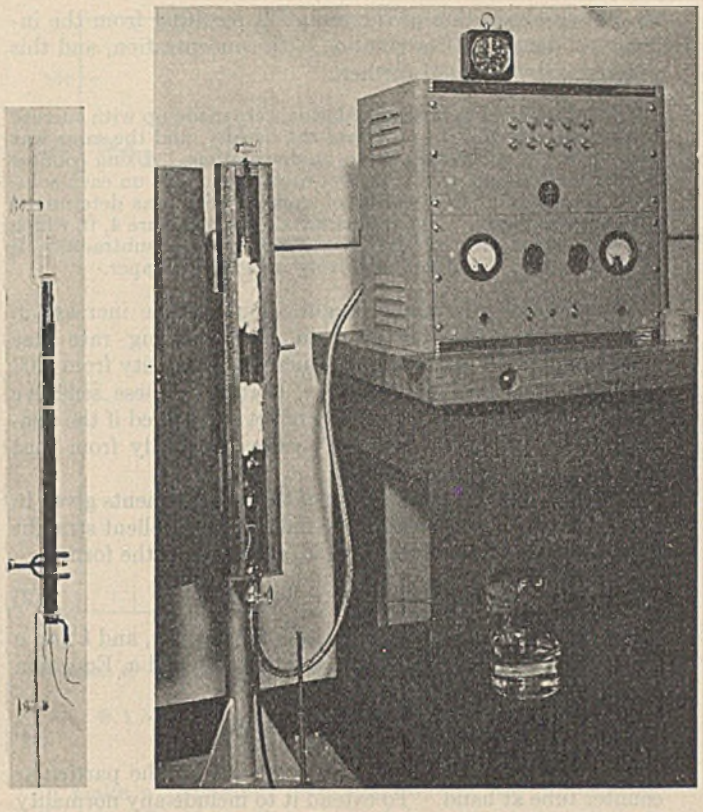

FIGURE 2
(*Left*) Large counter tube. (*Right*) Large counter tube and circuit

TABLE I. COMPARISON OF KNOWN AND CALCULATED POTASSIUM CONCENTRATIONS

Potassium Salt	Added Salt — Kind	Added Salt — Normality	Net Count per Minute	Potassium Normality — Known	Potassium Normality — Calculated
KCl	...	...	14.3	1.0	0.98
KCl	...	...	29.1	2.0	1.98
KCl	...	...	44.0	3.0	3.00
KCl	...	...	58.4	4.0	3.96
KCl	NaCl	3.0	14.8	1.0	1.01
KCl	NaCl	1.0	43.5	3.0	2.97
$K_4Fe(CN)_6$	...	...	14.1	1.0	0.96
$K_4Fe(CN)_6$	...	...	44.1	3.0	3.01
K_2CO_3	...	...	12.1	1.0	0.87
K_2CO_3	...	...	44.3	3.0	3.03
KCl	NaCl	0.68	17.0	1.07	1.16
	$Fe(NH_4)(SO_4)_2$	0.008			
	$CaCl_2$	0.18			
	NH_4Cl	0.38			

amplifier and scaling circuit was of conventional type with a simple circuit as the first stage. The cell for the solutions was a thin-walled annular glass vessel which fitted closely around the counter tube, and held about 20 cc. of solution.

The procedure adopted was to prepare solutions of known concentrations of various potassium salts, alone and mixed with other salts, and to make a 2-hour count on each. Background counts were taken for the same length of time with distilled water in the cell. (The background throughout these experiments remained very constant and was about 25 counts per minute.)

The data obtained for all the solutions measured are assembled in Table I. Examination of the data for 1, 2, 3, and 4 *N* potassium chloride solution indicates that the net count was directly proportional to the concentration. This is shown graphically in Figure 1, in which the best straight line is drawn through the points. For all the other solutions, therefore, the normality corresponding to an observed count could be read directly from the graph; these are the so-called "calculated normality" figures recorded in the last column of Table I. That the method has practical possibilities is obvious from the agreement of the known and calculated values.

The observation that the count was directly proportional to the concentration of potassium was to be expected from the original work on the activity of potassium. Of great importance for the purpose at hand was the fact brought out in Table I, that the nature of the accompanying anion or of added salts had such a small effect on the counting rate that no interference was apparent with this apparatus. While these results, therefore, showed the feasibility of performing potassium analyses by a radioactive method, the procedure was unsatisfactory because of the low sensitivity of the counter tube. Even for rather concentrated solutions, the observed count was only 15 to 60 counts per minute above the background. Consequently, excessively long times were required for any reasonable accuracy and samples of low concentration could not be determined successfully.

In order to show how sensitivity, accuracy, and time of analysis are interrelated, a short discussion may be inserted at this point.

The disintegration of a radioactive substance is a random process subject to statistical laws which can be deduced from probability considerations. Thus, in order to measure the activity of a material with absolute accuracy, a count of the number of particles disintegrating in an infinitely long time would be required. However, it may be shown that the probable error in a single set of counts for a given time interval is 0.67 of the square root of the number of counts observed. For example, if 10,000 counts have been made, the probable error in this measurement is 0.67 $\sqrt{10{,}000}$ or 67 counts, and the relative accuracy would be ± 0.67 per cent. For a relative accuracy of ± 1 per cent it would be necessary to count approximately 4500 emissions. The following table indicates the relationship between the probable error and minimum number of counts required for the stated accuracy:

Probable Error, %	Total Count Required
10	45
5	180
3	450
1	4,500
0.5	18,000
0.1	450,000

The presence of the background count introduces an additional source of error. Whenever the number of counts from a sample is so small that it approaches the background count, it is then necessary to count from five to ten times as many disintegrations to obtain a desired precision for the measurement. A further discussion of these points may be obtained from Rasetti (*11*) or from Strong (*14*).

The foregoing paragraph makes it clear that a more sensitive tube would extend the over-all accuracy of the method or decrease the time required for performing an analysis of a given accuracy. Inasmuch as this sensitivity of a counter tube is a function of its geometry and size, a new tube was designed to accomplish this.

Although similar to the first type in that it was of thin-walled glass silvered on the inside, it was considerably larger and had an outer jacket built around the counter tube proper. The annular space so formed held the solution to be counted, and could be filled by sucking liquid up through a lower inlet. With this design only one thin glass wall was interposed between the solution and counter interior. Tubes similar in principle but smaller in size have already been described (*7*, *10*). The tube is shown in Figure 2; on the right it is shown in place in its lead shield and with the amplifying circuit connected. The diameter of the inner tube is about 2.4 cm., the outer tube or jacket is 3.5 cm., and the spacing between the walls of jacket and inner tube is about 4 mm. The jacketed portion of the tube is about 45 cm. long, and the volume of the jacketed space is about 200 cc. The counter gas used is the usual 90-10 argon-alcohol mixture at a total pressure of 100 mm., and the tube is operated on the con-

ventional simple circuit. It showed excellent counting characteristics under these conditions with a threshold of about 900 volts and a plateau 100 to 140 volts long.

A calibration of two different tubes was run with standard potassium chloride solutions. Twenty-minute counts were made, and background was counted with distilled water in place of the potassium chloride solution.

The background count in the absence of distilled water is always higher than with water present. This effect was noted with all the tubes tried, and the cause is not explained. Possibly the water provides additional shielding from external radiation, perhaps from the lead itself It does not seem to be due to a natural radioactivity of the Pyrex, for counts of samples of Pyrex have shown no appreciable activity. In any event, the water is sufficiently dense to screen out all such external radiation, as a solution of zinc chloride of density 1.5 gave the same count as distilled water. Exposure to sunlight increases the counting rate, and consequently, if no lead housing is available, the tube at least should be covered with a black cloth.

The results are shown in Figure 3, in which counting rate is plotted against the normality of the potassium chloride. The background count has been included in these calibration curves in order to indicate the magnitude of the counts actually recorded.

The total sensitivity of the method was increased about 30-fold by using the larger tube, for the net count of 1 N solutions in the large counter was about 400 counts per minute as compared to 14 for the small one. Clearly, solutions as dilute as 0.1 to 0.05 N may be analyzed with fair accuracy by making rather long counts. For concentrations of 1 N or higher, the precision for a 20-minute count is about 0.01 N, as was shown by analyzing two unknown solutions:

	Potassium Normality	
	Known	By radioactivity
Unknown 1	2.274	2.26 ± 0.01
Unknown 2	1.186	1.18 ± 0.01

A closer examination of Figure 3 reveals the fact that for both new tubes the counting rate is not quite proportional to the concentration, but falls off with increasing potassium content. Such an effect was not observed with the smaller tubes, since their sensitivity was too low. The effect was ascribed to the self-absorption of the radiation resulting from the increase in density of the solution with concentration, and this point was investigated further.

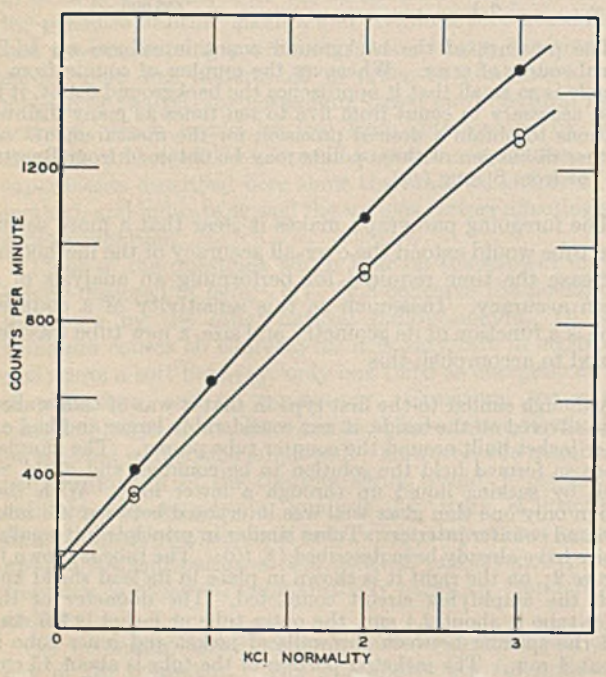

FIGURE 3. COUNTING RATE *vs.* POTASSIUM CONCENTRATION

● Large counter tube *B* ○ Large counter tube *A*

1 N solutions of potassium chloride were made up with sucrose or zinc chloride added to increase the density, and the same was done with 1 N potassium ferricyanide solutions. Using counter A, duplicate counts of 20 minutes each were made on each solution; in addition, the density of each solution was determined with a pycnometer. The results are shown in Figure 4, in which the net count per minute—i. e., background subtracted—is plotted against the observed density on semi-log paper.

The decrease in count brought about by the increase in density is obvious—for example, the counting rate was lowered about 10 per cent by an increase in density from 1.03 to 1.30. It is evident, therefore, that with these sensitive tubes a correction for the density effect is required if the density of the "unknown solution" varies markedly from that used in the calibration.

The method of representation of the measurements given in Figure 4 was chosen because the data fit an excellent straight line. The equation of this line, is, of course, of the form

$$\log (c/m) = k - (a)\ (d) \qquad (2)$$

where (c/m) is the counting rate, d is the density, and k and a are empirical constants. On evaluation of k and a, Equation 2 becomes:

$$\log (c/m) = 2.8525 - 0.234\ d \qquad (2)$$

This equation applies for 1 N solutions for the particular counter tube at hand. To extend it to include any normality of solution, the term log N is merely included, so that the equation reads

$$\log (c/m) = 2.8525 - 0.234\ d + \log N \qquad (3)$$

Now this equation not only fits the data of Figure 4 but also reproduces the calibration curve of counter tube A in Figure 3 within a precision of ±1 per cent. The fact that the numerical constants as obtained from the count-density curve of Figure 4 for 1 N solutions are applicable to potassium chloride solutions of any normality constitutes proof that the density is the factor responsible for the falling off of the counting rate with increased concentration, as observed in Figure 3.

A general equation of the form of (3)

$$\log (c/m) = k - (a)\ (d) + \log N \qquad (4)$$

applies for any counter tube, but the constants vary from tube to tube. For example, for tube B the equation as determined from the calibration curve in Figure 3 is

$$\log (c/m) = 3.1555 - 0.463\ d + \log N \qquad (5)$$

The value of k should clearly vary from counter tube to counter tube, for in effect k is the logarithm of the count which would be given by a 1 N solution of potassium at zero density. This must naturally depend on the length and wall thickness of the jacketed portion of the counter. The variation in the constant a from tube to tube may probably be due to the fact that the radiation from potassium is not homogeneous for both beta- and gamma-rays are effective in firing the counter, and furthermore, for potassium the beta-rays themselves show two different energy groups. The variation in thickness of the layer of solution from tube to tube may also play a role. It is an interesting point that an apparent half-thickness of about 1.4 grams per sq. cm. may be calculated from the slope of the curve of Figure 4. This lies between the value for the gamma-rays (about 5 grams per sq. cm.) and the beta-rays (about 0.03 gram per sq. cm.), and would suggest that the radiation actually entering the counter consisted of about 75 per cent beta-rays and 25 per cent gamma-rays, with the wall thickness of the tube about 0.2 to 0.4 mm.

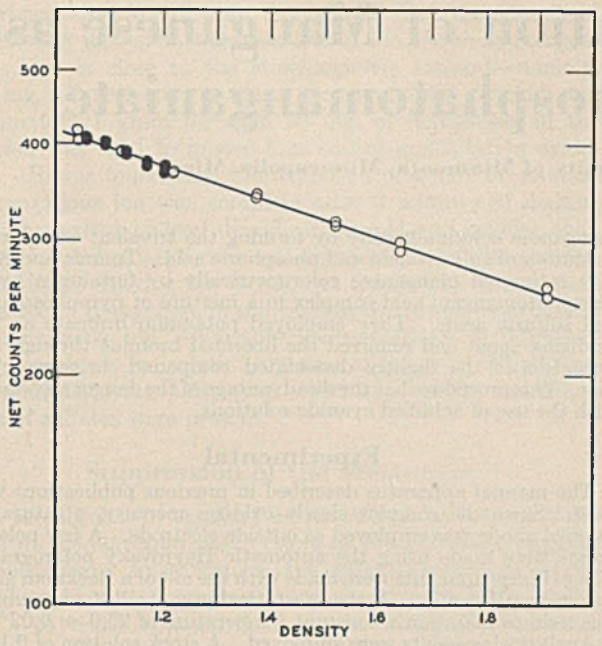

FIGURE 4. EFFECT OF DENSITY OF SOLUTION ON COUNTING RATE

● 1 *N* KCl + $ZnCl_2$ ○ 1 *N* $K_4Fe(CN)_6$ + sucrose

To make a potassium determination by the radioactive method described here, the counter tube to be used must first be calibrated. This can be done with only two solutions of potassium, say 1 *N* and 3 *N* potassium chloride. The observed counts per minute and the known densities of the calibrating solutions are substituted in Equation 4 and the constants k and a evaluated. The empirical relation between count, density, and normality is thus obtained for the counter at hand. For any unknown solutions, the counting rate and the density must be measured, the values substituted in the equation and the normality then calculated.

The above method of correcting for the effect of density is applicable when the element in the salt mainly responsible for the high density is of atomic number below zinc (Zn = 30). The density correction necessary if elements of higher atomic number are present may be as much as 50 per cent greater and seems to depend on the element. For example, in trials with potassium nitrate solutions with barium chloride or with lead nitrate added to make the density 1.33, the observed count was 94 and 90 per cent, respectively, of the count obtained when zinc chloride was used to bring the density of the potassium nitrate solution to the same value. In general, compounds with elements of such high atomic number will not be found in amounts sufficient to contribute predominantly to the density of the solution, so that the correction determined as previously described may be used safely. However, if salts of heavy metals are present in high concentration, these had best be removed before the counting of the potassium solution is undertaken. As an alternative procedure, the counter tube may be calibrated with potassium solutions increased in density by addition of the salt present in the unknowns.

On the basis of this work, an apparatus was installed for routine use in a control analytical laboratory and has been in operation for about 6 months. In that time, many analyses have been made which could have been carried out by the usual procedures only with far greater expenditure of time and labor.

Conclusions

The results presented in this report indicate that a rapid and comparatively simple determination of potassium can be made by taking advantage of its natural radioactivity. Obviously, the type of equipment used in making these analyses may be applied with advantage for work with other radioisotopes, whether natural or artificial. The size and design of the tubes employed naturally are not restricted to those described here, but may be altered to meet particular conditions. Once the apparatus has been set up, a determination requires 10 minutes to 2 or 3 hours, depending on the sensitivity of the counter, the potassium concentration, and the accuracy desired. The outfit itself is not too complicated and can be assembled at a total cost of about $450. Operation is simple, and maintenance of the apparatus is negligible, although the circuit may require adjustment occasionally. The life of the counter tube is indefinite, but usually the good counting characteristics, if lost, can be restored by pumping and refilling the tube with counter gas. The advantages of the method are obviously such as to recommend it for general use.

Acknowledgment

It is a great pleasure to acknowledge the help and advice of J. R. Dunning of Columbia University, J. W. Kennedy of the University of California, Berkeley, and R. C. Raymond of the Massachusetts Institute of Technology, in connection with the design of the counter circuit and of the tubes.

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Suspension of Glass Thermometers

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THE glass ring provided at the top of glass thermometers for suspension from a hook, wire, or string often breaks off in handling because of its rigidity. If the thermometer itself is still in working order, it is usually suspended through a rubber stopper or cork held in place by means of a buret clamp.

A better method is to fit a short piece of rubber tubing snugly over the upper end of the thermometer, so that about an inch of the tubing extends freely at the top. A small hole is cut in the end of the tubing by bending 0.25 inch of the top down sharply and making a very short lengthwise cut at the bend with a pair of scissors.

The thermometer can then be suspended freely in the usual manner. As a matter of fact, new thermometers may be thus provided. In this manner, the danger of contaminating a material with spilled mercury from a broken thermometer is considerably lessened.

Polarographic Determination of Manganese as Tri-dihydrogen Pyrophosphatomanganiate

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A procedure is presented by which manganese is quantitatively oxidized with lead dioxide to the trivalent state in a medium which contains a large excess of pyrophosphate at a pH smaller than 4. The concentration of the manganic manganese in the complex is determined polarographically.

Evidence is given that the violet complex ion is tri-dihydrogen pyrophosphatomanganiate.

THE present paper describes a simple procedure in which manganese is oxidized with the aid of lead dioxide to the manganic complex in the presence of a large excess of potassium pyrophosphate at a pH between 2.0 and 4.0. The concentration of manganic complex is determined with the aid of the dropping mercury electrode. Evidence that the complex has the composition tri-dihydrogen pyrophosphatomanganiate is given.

Manganous ion is reduced at the dropping mercury electrode, producing a well-defined diffusion current in neutral alkali chloride as supporting electrolyte (*14*). The half-wave potential is −1.51 volts *vs.* the saturated calomel electrode, so the presence of an excess of several metal ions such as ferric, ferrous, cobaltous, nickel, zinc, and copper interferes with the polarographic determination of divalent manganese. Furthermore, the hydrogen wave interferes in acidic solution. The permanganiate ion produces a poorly defined wave starting at zero applied e. m. f. Stackelberg *et al.* (*12*) concluded that this wave was not suitable for analytical purposes. Verdier (*14*) found that in an alkaline tartrate solution, a well-defined anodic wave was obtained at −0.4 volt *vs.* the saturated calomel electrode. The manganous ion is apparently oxidized to the trivalent state. A large excess of ferrous iron interferes in this determination. Tri-dihydrogen pyrophosphatomanganiate has the advantage that the manganese is reduced from the tri- to the divalent state at positive potentials *vs.* the saturated calomel electrode. As the diffusion current can be measured at +0.1 volt to +0.15 volt *vs.* saturated calomel electrode, it is not necessary to remove dissolved oxygen. A large excess of ions such as ferric, zinc, copper, cobalt, and nickel may be present. Chromium, vanadium, and cerium interfere, since these metals are oxidized to chromate, vanadate, and ceric, respectively, and are also reduced at positive potentials.

Trivalent manganese has been employed in several analytical methods for determining manganese.

Heczko (*7*) oxidized manganese to the trivalent state with freshly prepared perphosphoric acid in a solution of phosphoric acid and sulfuric acid and titrated the trivalent manganese iodometrically. Lang (*10*) oxidized manganese with potassium dichromate in a solution of metaphosphoric acid. After reducing the excess dichromate with sodium arsenite solution, he titrated the trivalent manganese with standard ferrous sulfate solution using diphenylamine as the indicator. Hirano (*8*) estimated manganese colorimetrically by forming the trivalent complex in a solution of sulfuric acid and phosphoric acid. Tomula and Aho (*13*) estimated manganese colorimetrically by forming a pyrophosphatomangani acid complex in a mixture of pyrophosphoric and sulfuric acids. They employed potassium bromate as the oxidizing agent and removed the liberated bromine through the formation of the slightly dissociated compound, bromine cyanide. This procedure has the disadvantage of the danger associated with the use of acidified cyanide solutions.

Experimental

The manual apparatus described in previous publications was used. Since the complex slowly oxidizes mercury, a saturated calomel anode was employed as outside electrode. A few polarograms were made using the automatic Heyrovský polarograph. The pH measurements were made with the aid of a Beckman glass electrode pH meter. A thermostatically controlled water bath was used to maintain a constant temperature of 25.0 ± 0.02° C.

Analytical reagents were employed. A stock solution of 0.1 *M* manganous sulfate was prepared by dissolving 22.306 grams of manganous sulfate tetrahydrate in water containing 10 ml. of approximately 1 *N* sulfuric acid and diluting to exactly 1 liter. The molarity was checked by a gravimetric determination in which the manganese was precipitated as manganous ammonium phosphate and weighed as manganous pyrophosphate. The molarity was found to be correct.

Source of Pyrophosphates

Although orthophosphoric acid may be converted to pyrophosphoric acid if heated at 215° C., large amounts of silica are dissolved if the conversion is performed in a Pyrex container. Platinum is also slowly attacked. Furthermore, concentrated pyrophosphoric acid is variable in composition (*5*) and difficult to handle because of its viscosity. Its aqueous solution is unstable, being completely hydrolyzed in a few days.

The authors investigated the use of a mixture of alkali pyrophosphates and strong mineral acids as the source of the dihydrogen pyrophosphate ion. Sodium pyrophosphate is only moderately soluble, 3.16 grams of the anhydrous salt dissolving in 100 ml. of water at 0° C. and 40.26 grams at 100° C. Potassium pyrophosphate is extremely soluble and is therefore more suitable for the preparation of a concentrated stock solution. Such a solution is stable for a long time if not acidified (*6*). The potassium pyrophosphate used in the following experiments was prepared by heating anhydrous dipotassium hydrogen phosphate in a furnace at from 500° to 700° C. for 3 hours.

Sodium pyrophosphate may be used instead of potassium pyrophosphate. In this case it is necessary to weigh out the salt for each determination.

Sulfuric and nitric acids were found satisfactory for the regulation of the acidity. Nitric acid is preferable if an appreciable amount of sulfate is present in the sample, since potassium sulfate, which is only moderately soluble, may precipitate.

Selection of an Oxidizing Agent

Lead dioxide was found to be a satisfactory oxidizing agent. It rapidly oxidized manganous ion to a violet-colored complex in a solution 0.4 *M* in pyrophosphates having a pH of 4 or less. The excess lead dioxide was removed by filtering through a retentive filter paper, such as Whatman No. 42. By means of titrations with standard ferrous sulfate solution it was shown that the oxidation was quantitative. It was

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experimentally found that 1 gram of lead dioxide could oxidize 388 mg. of manganous ion to the trivalent complex ion. This is close to the stoichiometric amount—namely, 460 mg. Practically, a large excess of lead dioxide, approximately 1 gram for each 50 mg. of manganese in the solution was used to hasten and ensure quantitative oxidation. It was found that the oxidation of a 0.01 *M* solution of manganous ion was complete after 3 minutes of shaking with this excess of lead dioxide if the pH was smaller than about 4.0 and a large excess of pyrophosphate was present. Intermittent shaking for 10 minutes resulted in quantitative oxidation of manganese in solutions as concentrated as 0.02 *M* in manganous ion. Variable concentrations of plumbous lead remained in solution, presumably as a dihydrogen pyrophosphato complex, even when appreciable concentrations of sulfates were present.

Suppression of the Maximum

In the absence of capillary-active substances a large maximum in the current-voltage curve of the manganic complex was obtained. Furthermore, the galvanometer oscillations were irregular at potentials more negative than the region (+0.1 to +0.3 volt *vs.* S. C. E.) in which the maximum occurred. These disturbances persisted in the presence of various agents generally used for the elimination of maxima. Gelatin, starch, soap, tylose, camphor, thymol, methyl orange, tropeolin 00, methyl red, and methylene blue were unsatisfactory in the suppression of the maximum.

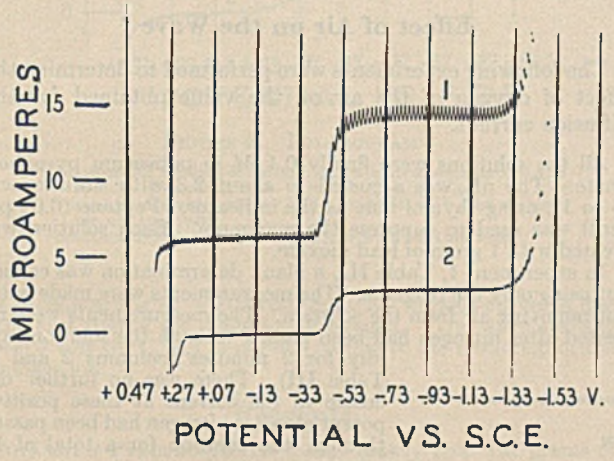


FIGURE 1. POLAROGRAM

1. 5 millimolar tri-dihydrogenpyrophosphatomanganiate, 0.4 *M* sodium pyrophosphate, 0.8–0.9 *M* nitric acid, 0.1 per cent in gum arabic, pH = 2.0; oxidized with lead dioxide; hydrogen passed through solution 15 minutes
2. Like 1 except no manganese present

It was found that if the solution was 0.02 per cent in agar the galvanometer oscillations became regular and diffusion current measurements could be made at potentials more negative than +0.16 volt (S. C. E.) using the manual polarograph. Many of the quantitative measurements were made with the manual apparatus, using agar to suppress the maximum. However, when polarographic determinations were attempted using the automatic Heyrovský polarograph, agar was found unsatisfactory. Temporary irregularities, which did not interfere when the manual instrument was used, were frequently recorded in the polarogram.

It was then found that if the final solution was 0.04 per cent in peptone (Merck's, dried, from meat) excellent waves were obtained with either the manual apparatus or the polarograph. It was necessary to add the peptone prior to the oxidation by lead dioxide. If added after the oxidation, a small amount of the complex was reduced.

After the experimental work was concluded the authors found that 0.1 per cent gum arabic is just as effective as peptone. It is stable for a long time and does not decompose the complex if added after the oxidation.

Within experimental error, the same diffusion current per millimolar concentration of the manganic complex was obtained whether 0.05 to 0.2 per cent agar, 0.02 to 0.1 per cent peptone, or 0.1 per cent gum arabic was used to eliminate the maximum.

The procedure followed in the preliminary experiments consisted of preparing solutions containing manganous ion and various concentrations of pyrophosphate at various pH values and in the presence of various maximum suppressors. The manganese was oxidized by shaking intermittently with lead dioxide for 5 to 10 minutes and the solution was filtered. Current voltage measurements were made using a portion of the filtrate. The removal of air is not necessary, since the diffusion current of the manganic complex is obtained before the oxygen wave starts.

Figure 1 illustrates the characteristics of the wave.

The solution used to obtain curve 1 was 0.005 *M* in manganic manganese, 0.4 *M* in sodium pyrophosphate, 0.8 to 0.9 *M* in nitric acid, and contained 0.1 per cent gum arabic. The pH was 2.00. The solution used to obtain curve 2 contained the same constituents, except that no manganese was added. The solutions were treated with lead dioxide as explained above. Tank hydrogen was passed through the solution in the cell for 15 minutes to remove most of the oxygen. In curve 2 the current from +0.2 to −0.4 volt (S. C. E.) was the residual or condenser current and was practically zero at −0.1 volt (S. C. E.). The anodic wave just beyond +0.25 volt is due to the dissolution of mercury. This wave begins at a lower positive potential than in the corresponding polarograms in Figures 2 and 3, owing to the presence of chloride ion introduced by prolonged contact with the saturated calomel anode. The wave beginning at −0.4 volt (S. C. E.) is due to the reduction of the plumbous lead introduced as a result of shaking with lead dioxide. The wave starting at −1.3 volt (S. C. E.) is due to the reduction of hydrogen ions. In curve 1, the wave beginning at about +0.3 volt is due to the reduction of the trivalent manganic complex to the divalent state. The second wave for the reduction of plumbous lead is larger than in curve 2 as a consequence of the reduction of more lead dioxide in the oxidation process. The true diffusion current is the difference between the magnitude of the apparent diffusion current measured and the residual current measured at the same potential, using a blank solution containing no manganese or a portion of the original solution before treatment with lead dioxide.

The half-wave potential of the wave due to the reduction of the manganic complex is obviously not a function of the standard potential of the manganous complex–manganic complex couple. The potential of a platinum electrode in a similar solution in which one-half of the complex had been reduced with ferrous sulfate solution was about +0.78 volt (S. C. E.) when the pH was 2.06.

Effect of pH

The pH may be of influence for several reasons. From the Nernst equation it is readily seen that the oxidation potential of lead dioxide is a function of the pH. Theoretically the oxidation potential is decreased by approximately 0.12 volt for each unit increase in pH. The kind of acid pyrophosphate which preponderates is determined by the pH, as is the stability of complex ions containing ionizable hydrogen atoms. It will be shown in a subsequent paper that the wave due to the reduction of ferric iron in pyrophosphate solutions is shifted to more negative potentials if the acidity is decreased. This wave interferes if the pH is much smaller than 2.

Experiments 1 to 8, Table I, were performed to determine

TABLE I. EFFECT OF pH

Sample, 5 ml. 0.1 *M* $MnSO_4$ to produce a final concentration of 5 millimolar in manganese.
Procedure followed, except pH varied. Final pyrophosphate concentration was 0.4 *M*, agar 0.04 per cent.
Capillary I. $m^{2/3}t^{1/6}$ = 1.314 mg.$^{2/3}$ sec.$^{-1/2}$ at $E_{d.e.}$ = +0.1 volt. Av. i_d/millimolar concn. Mn^{III} = 1.54 microamperes.

Experiment No.	H_2SO_4 (1:1) Used	pH of Final Solution	Color of Final Solution	i_d at +0.1 Volt (S. C. E.) Corrected for Residual Current
	Ml.			*Microamperes*
1	2	....	Brown	7.34
2	4	5.05	Orange brown	7.64
3	4	5.00	Orange brown	7.69
4	4.5	3.78	Violet	7.78
5	5	1.90	Violet	7.82
6	5	1.90	Violet	7.78
7	10	0.90	Violet	7.79
8	20	0.10	Violet	7.84

the pH range in which the violet complex could be quantitatively obtained with a large excess of pyrophosphate present.

Samples containing 5 ml. of 0.1 *M* manganous sulfate were transferred to a 100-ml. volumetric flask, and 20 ml. of 2 *M* potassium pyrophosphate were added to each flask to obtain a 0.4 *M* concentration of pyrophosphate. Various volumes of sulfuric acid (1 to 1) were added to regulate the pH. After addition of 2 ml. of 2 per cent agar and dilution to 100 ml., the oxidation and diffusion current measurements were carried out as in the general procedure.

All the solutions having a pH of 3.78 or smaller had, within experimental error, the same diffusion current and the rich violet color. However, in experiments 2 and 3, the solutions having a pH close to 5 appeared orange red to orange brown in color and the diffusion current was somewhat smaller than at lower pH. After completing the experiments, the pH of the solution used in experiment 4 was varied by adding nitric acid or ammonium hydroxide dropwise. When the pH was increased to 4.52, an orange cast was observed. When the pH was decreased below 4, the complex again appeared violet. A brown precipitate of manganese dioxide always formed in the brown solutions upon standing a few hours. The violet solutions were unchanged after 48 hours.

Effect of Pyrophosphate Concentration

A series of experiments was performed in which the pH was maintained close to 4.0 while the concentration of pyrophosphate was varied. The same final concentration of manganese, 0.005 *M*, was employed; 0.02 per cent agar was used to suppress the maximum. Before making the polarographic measurements in experiments 1 through 13 in Table II, sufficient potassium nitrate was added to 40 ml. of the oxidized solutions to make the concentration of potassium ions in all solutions 1.6 *M*. The solutions were then diluted to 50 ml. The oxidation to the trivalent complex was not complete until the pyrophosphate concentration was 0.2 *M* or 40 times larger than that of manganese.

Experiments 14 through 17 were then performed to determine the maximum concentration of manganese which could be oxidized to the trivalent state, using a final concentration of 0.4 *M* potassium pyrophosphate. Since a constant large excess of potassium pyrophospate was present, no addition of potassium nitrate or dilution was necessary before making diffusion current measurements. Experiment 14 showed that 10 millimolar manganous ion was quantitatively converted to the trivalent complex at pH 3.9. However, in experiment 15, when the concentration of manganese was increased to 20 millimolar, only 90 per cent of the manganese was converted to the trivalent complex at pH 3.3.

In experiments 16 and 17, 20 millimolar manganous ion and 0.4 *M* potassium pyrophosphate were again employed but the acidity was increased. In experiment 16, in which the pH was 2.2, a quantitative oxidation to the manganic complex was obtained. In experiment 17, in which a 7 *N* excess of free sulfuric acid was present, the oxidation to the trivalent complex was also quantitative. However, the complex was not stable in strong sulfuric acid. After 5 days the violet color of the complex prepared in 7 *N* sulfuric acid had disappeared, while the complex prepared at pH 2.2 showed no appreciable decrease in color.

It may be concluded that manganous ion, up to a concentration of 20 millimolar, can be quantitatively oxidized to the violet manganic complex in a solution 0.4 *M* in potassium pyrophosphate at a pH of about 2.2. At appreciably lower pH the complex is not very stable and the wave due to the reduction of the ferric complex interferes.

Effect of Air on the Wave

The following experiments were performed to determine the effect of oxygen of the air on the value obtained for the diffusion current.

All the solutions were finally 0.4 *M* in potassium pyrophosphate. The pH was adjusted to about 2.3 with sulfuric acid (1 to 1), using thymol blue as the indicator. Peptone (0.04 per cent) was used to suppress the maximum. Each solution was treated with 1 gram of lead dioxide.

In experiment 1, Table III, a blank determination was carried out using only the reagents. The measurements were made without removing air from the solution. The measurements were repeated after nitrogen had been passed through the solution rapidly for 2 minutes (columns 2 and 3, Table III). There was no further decrease in the current at these positive potentials after nitrogen had been passed through the solution for a total of 10 minutes.

In experiment 2, the procedure used in the previous experiment was followed but the solution was also 0.004 *M* in manganous ion before the oxidation. No reduction of oxygen was found at +0.15 volt (S. C. E.). The small value of 0.03 microampere at +0.1 volt (S. C. E.), due to reduction of oxygen, was measured in both the blank and the solution containing manganese. At 0.0 volt (S. C. E.) the effect due to oxygen was no longer the same in the blank and the solution containing manganese. It follows that it is not necessary to remove oxygen if the diffusion current is measured at +0.1 to +0.15 volt (S. C. E.).

These measurements using the manual instrument were substantiated with polarograms obtained with the Heyrovský polarograph. The polarogram (curve 2, Figure 2), was made without oxidizing the manganese and without removing the air. Another portion of the same solution was oxidized with lead dioxide to obtain curve 1. Air was not removed.

TABLE II. EFFECT OF PYROPHOSPHATE CONCENTRATION

Sample, 5 to 20 ml. 0.1 *M* $MnSO_4$ to produce a final concentration of 5 to 20 millimolar manganese.
Procedure followed except final pyrophosphate concentration varied. Agar, 0.04 per cent, used to suppress maximum. In experiments 1 through 13, 40 ml. of sample diluted to 50 ml. after oxidation and KNO_3 added to obtain 1.6 *M* potassium ion. Experiments 14 through 17 were not diluted.
Capillary II. $m^{2/3}t^{1/6}$ = 1.967 mg.$^{2/3}$ sec.$^{-1/2}$ at $E_{d.e.}$ = +0.1 volt. Av., i_d/millimolar concn. Mn^{III} = 2.30 microamperes.

Experiment No.	2 *M* Potassium Pyrophosphate Added	Final Millimolar Pyrophosphate Concn.	Final Millimolar Concn. of Mn	Final pH	Color of Final Solution	True Diffusion Current at +0.1 Volt (S. C. E.) (i_d Appparent$-i_r$)	Estimation of Mn Oxidized to Trivalent State
	Ml.					*Microamperes*	%
1	0.5	10	5	3.30	Violet brown	3.55	39
2	0.75	15	5	3.30	Violet brown	3.85	42
3	1.00	20	5	3.30	Violet brown	4.10	45
4	1.25	25	5	3.90	Brown violet	4.50	49
5	1.50	30	5	4.09	Brown violet	5.67	62
6	1.75	35	5	4.11	Rose violet	6.37	69
7	2.0	40	5	4.00	Violet	7.19	78
8	3.0	60	5	3.98	Violet	7.50	82
9	4.0	80	5	4.03	Violet	7.85	85
10	5.0	100	5	3.78	Violet	8.35	91
11	7.5	150	5	3.98	Violet	8.82	96
12	10.0	200	5	3.90	Violet	9.18	100
13	20.0	400	5	3.90	Violet	9.17	100
14	20.0	400	10	3.90	Violet	23.0	100
15	20.0	400	20	3.30	Violet	41.4	90
16	20.0	400	20	2.30	Violet	46.2	100
17	20.0	400	20	7 *N* H_2SO_4	Violet	46.3	100

TABLE III. EFFECT OF AIR ON THE WAVE

Solution 0.004 *M* Mn, 0.4 *M* $K_4P_2O_7$, 0.08 per cent peptone, pH = 2.3 (approx.) using H_2SO_4 (1.1) and thymol blue.
Capillary III. $m^{2/3}t^{1/6}$ = 2.150 mg.$^{2/3}$sec.$^{-1/2}$ at *Ed. e.* = +0.1 volt.

	Experiment 1, No Mn Present			Experiment 2, 4 Millimolar Mn^{III}		
Ec (S. C. E.)	Air not removed	N_2 passed through solution 2 minutes	Current due to air in blank	Air not removed	N_2 passed through solution 2 minutes	Current due to air in Mn solution
	Microamperes					
+0.25	−4.35	−4.35	0	4.30	4.30	0
+0.24	−2.86	−2.86	0	5.55	5.55	0
+0.22	−0.56	−0.56	0	....	....	....
+0.20	−0.38	−0.38	0	9.43	9.43	0
+0.15	−0.20	−0.20	0	9.65	9.65	0
+0.1	−0.13	−0.16	+0.03	9.84	9.81	+0.03
+0.075	0.00	....	....	9.89	9.84	+0.05
+0.05	+0.13	−0.10	+0.23	9.98	9.86	+0.12
+0.025	+0.24	....	....	10.06	9.88	+0.18
0.00	+0.41	−0.06	+0.47	10.16	9.90	+0.26

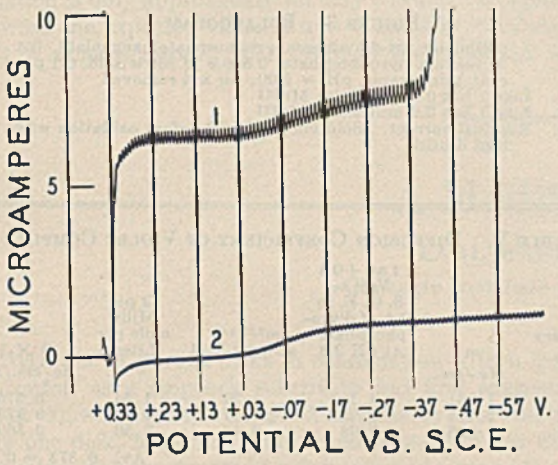


FIGURE 2. POLAROGRAM

1. 5 millimolar tri-dihydrogenpyrophosphatomanganiate, 0.4 *M* sodium pyrophosphate, 0.8–0.9 *M* nitric acid, 0.1 per cent gum arabic, pH = 2.00; oxidized with lead dioxide; air not removed
2. Solution used in 1 before oxidation with lead dioxide

The wave starting at +0.05 volt (S. C. E.) in both curves is due to the first step of the reduction of oxygen. The wave starting at −0.4 volt (S. C. E.) in curve 1 is due to the reduction of the plumbous ion.

Reagents

POTASSIUM PYROPHOSPHATE, 2 *M*. Slowly pour 132 grams of anhydrous potassium pyrophosphate into about 150 ml. of water while stirring. Dilute to exactly 200 ml.

NITRIC ACID (1 to 1). Dilute 100 ml. of freshly boiled concentrated nitric acid with 100 ml. of water.

SULFURIC ACID (1 to 1). Dilute 100 ml. of concentrated sulfuric acid with 100 ml. of water.

GUM ARABIC, 2 per cent. Add 1 gram of gum arabic to 49 grams of water and heat to boiling while stirring. This solution is stable for several days.

PEPTONE, 2 per cent. Dissolve 1 gram of Merck's peptone, "dried", from meat, in 49 grams of boiling water. This solution may be kept in a stoppered bottle containing a few milliliters of carbon tetrachloride, kept cool, or prepared fresh after 48 hours.

THYMOL BLUE SOLUTION, 0.1 per cent. Mix 0.1 gram of dry thymol blue with 21.5 ml. of 0.01 *N* sodium hydroxide in a mortar and dilute to 100 ml. with water.

Procedure

POLAROGRAPHIC DETERMINATION OF MANGANESE AS TRI-DIHYDROGEN PYROPHOSPHATOMANGANIATE. The sample to be determined may contain from 1 to 100 mg. of manganese as the nitrate, sulfate, or perchlorate in approximately 50 ml. of aqueous solution. Iron may be present in quantities up to 0.2 gram, but chromium, vanadium, and cerium interfere.

Transfer the sample quantitatively to a 100-ml. volumetric flask, and add enough nitric acid (1 to 1) or sulfuric acid (1 to 1) to make the total amount of mineral acid present equivalent to 12 ml. of nitric acid (1 to 1) or 4.5 ml. of sulfuric acid (1 to 1). Slowly add 20 ml. of 2 *M* potassium pyrophosphate to the solution while swirling. Add 2 or 3 drops of thymol blue. Add more acid or ammonium hydroxide dropwise until the color becomes orange, corresponding to a pH of 2.0 to 2.4. Add 5 ml. of 2 per cent gum arabic or 5 ml. of 2 per cent peptone. Dilute to the mark and mix thoroughly.

Transfer a few milliliters to a polarographic cell and measure the residual current as described below for the oxidized solution. Add about 1 gram of manganese-free lead dioxide for each 25 to 50 mg. of manganese present and shake intermittently for 5 to 10 minutes. Filter through a dry retentive filter paper such as Whatman No. 42 into a clean polarographic cell. Rinse out the cell with the first portion of the filtrate and collect a suitable volume of the filtrate.

Use an external anode. Five milliliters of C. P. carbon tetrachloride may be added to cover the mercury drops collecting at the bottom of the cell. Just before starting the measurements insert the dropping mercury electrode. Measure the current at +0.1 volt and +0.15 volt (S. C. E.) if the manual instrument is used.

If the automatic recording instrument is available, use the 2-volt accumulator. Turn the switch to "anodic and cathodic polarization". The applied potential is zero when the sliding contact is at the exact center of the resistance wire. Regulate the voltage so that each abscissa line corresponds to 0.1 or 0.05 volt. Choose a galvanometer sensitivity such that the wave has a suitable height from +0.1 to +0.15 volt (S. C. E.). Start the polarogram at +0.35 volt (S. C. E.). If iron is present, stop the motor as soon as the light beam moves past the camera, then with the sliding contact disengaged record the zero current line for a distance of 1 or 2 mm. on the sensitized paper. It is desirable to run the residual current on the same polarogram.

TABLE IV. PROPORTIONALITY BETWEEN DIFFUSION CURRENT AND CONCENTRATION OF MANGANESE

Capillary I $m^{2/3}t^{1/6}$ = 1.314 mg.$^{2/3}$ sec.$^{-1/2}$ at *Ed. e.* = +0.1 volt.
Procedure used except 0.02 per cent agar used and nitrogen bubbled through solution 2 minutes.

Experiment No.	Final Millimolar Concn. of Complex Manganic Ion	Mn Taken	Apparent Diffusion Current Measured at +0.1 Volt	True Diffusion Current (Corrected for Residual)	Diffusion Current per Millimolar Concentration of Complex
		Mg.	*Microamperes*	*Microamperes*	*Microamperes*
1	0	0	−0.07	....	....
2	0	0	−0.07	....	....
3	0.2	1.1	+0.25	0.32	1.60
4	0.5	2.75	0.69	0.76	1.52
5	1	5.49	1.46	1.53	1.53
6	1	5.49	1.47	1.54	1.54
7	2	10.99	3.02	3.01	1.54$_4$
8	3	16.48	4.57	4.64	1.54$_7$
9	5	27.47	7.68	7.75	1.55$_2$
10	10	54.93	15.40	15.47	1.54$_7$
11	10	54.93	15.32	15.39	1.53$_8$
12	20	109.86	30.70	30.77	1.53$_8$
				Av.	1.54

Proportionality between Diffusion Current and Concentration of Manganese

The results reported in Table IV show that proportionality exists between diffusion current and the concentration over a wide range of concentrations.

The deviation from the average was more than 1 per cent only when very low concentrations of manganese were used.

The determinations were made according to the general procedure. A few polarograms are given in Figure 3. Even at very low manganese concentrations, oxygen from the air does not interfere.

Structure of Mangani Pyrophosphoric Acid

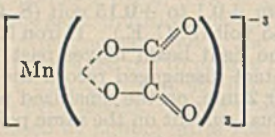
If manganic orthophosphate is dissolved in meta, pyro, or sirupy orthophosphoric acid, violet-colored complex compounds are formed. These compounds have been named mangani metaphosphoric acid, mangani pyrophosphoric acid, and mangani orthophosphoric acid, depending on the acid in which they were prepared.

A study of the literature reveals that the following types of trivalent manganese compounds have been crystallized from solutions of the various acids:

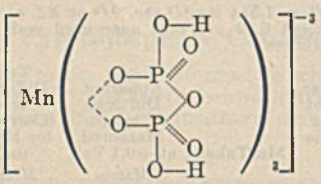
1. From orthophosphoric acid
   $MnPO_4.H_2O$, green gray in color (*4*)
2. From pyrophosphoric acid
   $Mn_4(P_2O_7)_3.14H_2O$, pale violet (*1*)
   $NaMn(P_2O_7).5H_2O$, pale red (*11*)
3. From metaphosphoric acid
   $Mn(PO_3)_3.H_2O$, red (*1*)

Rosenheim (*11*) observed that many compounds of the type M^1, $MnP_2O_7.3H_2O$, crystallized from solutions containing the mangani pyrophosphoric acid. He suggested the formula $[Mn\ {}^{P_2O_7}_{(H_2O)_3}]^-$ for the anion of the violet complex acid in aqueous solution. This formula has been adopted in the literature. The assumption of similarity between the complex in solution and the crystalline form obtained is not justified.

The violet manganic complex with pyrophosphate described in this paper has properties which are very similar to that of the trioxalatomanganiate complex. The latter has the following structure:



which is stabilized by the five-membered chelate ring. A similar structure with a six-membered chelate ring is obtained when the dihydrogen pyrophosphate ion combines with manganic:



Various observations substantiate the conclusion that the dissolved violet complex dealt with in this paper is the tri-dihydrogen pyrophosphatomanganiate of the above structure.

The pH of an alkali dihydrogen pyrophosphate solution is about 4.25. As is evident from Table I, the violet complex is stable at this and lower pH. In order to obtain a stable solution of the violet complex it is necessary to have present a large excess of dihydrogen pyrophosphate. This indicates that the violet complex has ionic bonding and that it has a relatively small stability constant. Cartledge and Nichols (*3*) in their study of trimalonatomanganiate arrived at a similar conclusion.

When the pH is of the order of 5 or larger dihydrogen pyrophosphate ions in the complex may be replaced by water molecules, producing the brown-colored complex. This behavior has been observed by Cartledge *et al.* (*2, 3*) in the study of trimalonatomanganiate and the trioxalatomanganiate complexes.

Tomula and Aho (*13*) found in transport measurements that the violet manganic pyrophosphate complex migrates toward the anode. This observation eliminates the possibility that pyrophosphoric acid occupies the coordination

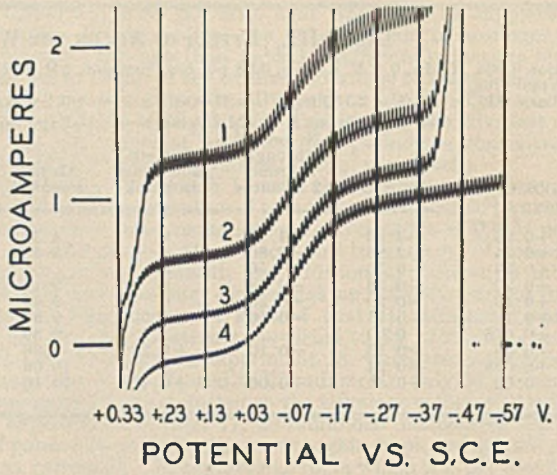


FIGURE 3. POLAROGRAM

1. 1 millimolar tri-dihydrogenpyrophosphatomanganiate, 0.4 *M* sodium pyrophosphate, 0.8–0.9 *M* nitric acid, 0.1 per cent gum arabic, pH = 2.00; air not removed
2. Like 1 but 0.5 millimolar Mn^{III}
3. Like 1 but 0.2 millimolar Mn^{III}
4. Residual current. Solution used in 3 before oxidation with lead dioxide

TABLE V. DIFFUSION COEFFICIENT OF VIOLET COMPLEX

Capillary No.	m	i at +0.1 Volt *vs.* S. C. E. in 0.4 *M* Pyrophosphate at pH 2.3	$m^{2/3}t^{1/6}$ at +0.1 Volt	i_d per Millimole per Liter	$D \times 10^5$
	Mg./sec.				*Sq. cm./sec.*
I	1.051	4.10	1.31	1.54	0.375
II	2.111	2.92	1.97	2.30	0.373
III	2.625	2.08	2.15	2.50	0.369
				Av.	0.373 ± 0.003

places in the complex. The possibility that trihydrogen pyrophosphate ions occupy the coordination positions is not considered probable, because an alkali trihydrogen pyrophosphate solution has a pH of about 1.40 and the complex was found stable at a pH of 4. Evidence that the complex is tri-dihydrogen pyrophosphatomanganiate was obtained from the calculation of the diffusion coefficient of the complex ion. The diffusion coefficient was calculated from the polarographic results with the aid of the Ilkovic equation:

$$i_d = 605\ n\ C\ D^{1/2}m^{2/3}t^{1/6}$$

By titration with ferrous sulfate it had been found that the manganese in the complex is present in the trivalent form. Hence n in the above equation is equal to one, assuming that the complex ion contains one manganic ion.

The diffusion coefficient was calculated from three sets of measurements with three different capillaries. The results are summarized in Table V.

For the sake of comparison the authors have also calculated the diffusion coefficient of the trioxalatocobaltiate ion from its diffusion current using capillary I. The medium was 1 *M* in potassium oxalate, 0.2 *M* in ammonium acetate, and 0.5 *M* in acetic acid, and contained 0.018 per cent gelatin. The solution had a pH of 5.0. The average value of the diffusion coefficient calculated was 0.523×10^{-5} sq. cm. per second.

Using Jander's (*9*) expression for the relation between the molecular weights and diffusion coefficients of two closely related species x and k:

$$M_x = \left(\frac{Z_kD_k}{Z_xD_x}\right)^2 M_k$$

in which Z is the specific viscosity of the solution, D, the diffusion coefficient, and M the molecular weight, it is possible to calculate the molecular weight of the manganic pyrophosphate complex. The viscosities of the media in which the diffusion currents of the manganiate and cobaltiate complexes were determined were nearly identical.

The formula of the oxalatocobaltiate has been proved (*2*) to be $Co(C_2O_4)_3^{---}$, corresponding to a molecular weight of 323. Substituting the various values in the above equation we find:

$$M_x = \frac{(5.23 \times 10^{-6})^2}{(3.75 \times 10^{-6})^2} \times 323 = 628$$

Assigning to the manganic dihydrogen pyrophosphate complex the formula $[Mn(H_2P_2O_7)_n^{3-2n}]$ we calculate from the molecular weight of 628 a value of $n = 3.25$. Since Jander's equation is only approximate we may consider the agreement between the expected value of $n = 3$ and the calculated one of 3.25 as satisfactory. The result of the molecular weight calculation indicates that the complex has the formula $[Mn(H_2P_2O_7)_3]^{---}$.

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A Modified Stock Valve

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THE use of porous disks in combination with a mercury cutoff as a stopcock substitute was first suggested by Stock (*2*), who used two disks. A modified type of valve using only one disk, to permit more rapid passage of gas through the valve, was constructed by Schumb and Crane (*1*).

The valve described uses one sintered-glass disk and has been designed to have a minimum of dead-end space and to withstand a pressure difference of one atmosphere in either direction without use of a barometric height of mercury. It has been used for pure gases only. Passage of a gas mixture with components of widely different molecular weights through the valve might lead to changes in the composition of the mixture. This new valve is more difficult to construct than the design of Schumb and Crane and is not so suitable for controlling the flow of liquids.

The construction of the valve is obvious from Figure 1. The sintered disk must have pores sufficiently fine so that mercury will not flow through the disk at the pressure differences which usually exist across the valve. If the valve is to withstand a pressure difference of one atmosphere, the commercially available disks with the smallest pores (graded as either D or F) should be used. For pressure differences lower than one atmosphere, coarser disks may be employed advantageously as they offer a lower resistance to the flow of gas through the valve. The sintered disk was obtained already sealed in a glass tube. In making seals near the sintered disk, the tubing and the disk should be heated slowly and uniformly. It is convenient to make the tubes leading to the mercury reservoir so long that when the line is evacuated, all the mercury in the valve can be withdrawn with a pressure of 30 mm. in the reservoir. Then a water aspirator may be used to operate the valve.

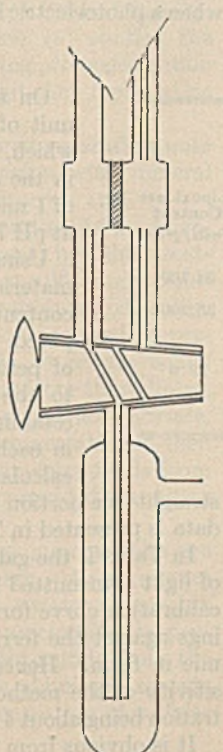

FIGURE 1

To close the valve, mercury is admitted to the compartment which is at the higher pressure. The gas pressure forces the mercury against the sintered disk and prevents flow of gas through the disk. If the pressure on one side of the valve fluctuates during the experiment above and below the pressure on the other side, mercury should be placed in both compartments. When both compartments have been filled with mercury, and the valve is to be opened, mercury should be withdrawn first from the compartment which is at the lower pressure. This procedure avoids rapid bubbling of the gas through the mercury and the possible spraying of liquid mercury through the line.

This valve possesses the advantages and the disadvantages of a mercury cutoff, such as freedom from leaks, no contact between gas and stopcock lubricant, and contamination of the gas with mercury vapor. In comparison with the conventional mercury cutoff, it is more compact and requires less mercury. However, the sintered disk offers considerable resistance to gas flow. To measure the magnitude of this effect, a series of flow tests was made on air using a 20-mm. Pyrex disk, No. 39570 F. The volume of air flowing through the disk in definite time intervals was measured with a wet gas meter, and the pressure difference across the disk was obtained with a dibutylphthalate manometer. The pressure difference across the disk was varied over the range 3.4 to 0.5 mm. of mercury. The experimental rate of flow was approximately constant over the given pressure range and was 6.5 cc. of air (25°, 1 atmosphere) per minute per mm. of pressure difference. As the measurements were made at a pressure near 1 atmosphere, it may be assumed that the pore diameter is large, relative to the mean free path of the gas molecules and that the flow is isothermal. Calculations show that a disk 2 mm. thick is equivalent to a capillary tube 2 mm. long and 0.16 mm. in diameter. Obviously, evacuation will take longer with this valve in the vacuum line than with the usual stopcock. The speed of evacuation may be increased by using larger disks. However, for kinetics investigations, traces of oxygen or other unwanted gases can be removed by flushing the reaction cell five or six times with the reactant gas. The flushing procedure is just as effective and takes much less time than prolonged evacuation.

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Lipoid Oxidase Studies

A Method for the Determination of Lipoxidase Activity

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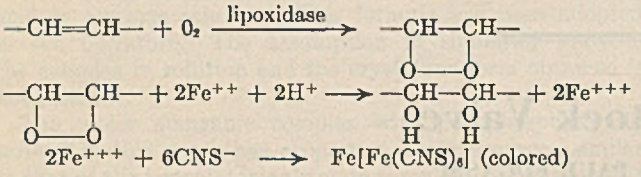
THE work of Sumner and Sumner (*4*) proved that the bleaching of carotene by the enzyme "carotene oxidase" is an indirect result of the action on unsaturated fats of a fat-peroxidizing enzyme. The existence of this lipoxidase had been noted by a few workers (*1*, *2*, *3*).

A method for the quantitative determination of enzymic peroxidation has been devised. It was found that the fat peroxides in dilute water suspension oxidize ferrous iron, and the ferric iron formed can be determined colorimetrically as the thiocyanate, according to the following reactions (*5*):



Details of Method

In a clean, dry 250-cc. Erlenmeyer flask are placed 5 mg. of linoleic acid dissolved in 5 cc. of acetone, and 100 cc. of water and 5 cc. of citrate buffer (pH 6.5) are added. [The buffer was made by mixing 55 cc. of a solution of citric acid (21.008 grams per liter) and sodium hydroxide (8 grams per liter) with 45 cc. of 0.1 *N* sodium hydroxide.] This mixture is brought to 25° C., and 1 cc. of aqueous enzyme extract is added and allowed to react for the desired time, the flask being rotated to produce adequate agitation. Ten cubic centimeters of concentrated hydrochloric acid are added to stop the reaction and to provide an acid medium for the oxidation of the ferrous iron by the fat peroxide, and 1 cc. of a 5 per cent solution of ferrous ammonium sulfate in 3 per cent hydrochloric acid is added. Either of the procedures discussed below may then be used.

TABLE I. DETERMINATION OF LIPOXIDASE CONTENT

Enzyme Source	Galvanometer Reading	Fe^{+++}	Net Fe^{+++}	Total Fe^{+++}	Time of Reaction	Weight of Enzyme Source	Lipoxidase Content
		γ/cc.	*γ/cc.*	*γ*	*Min.*	*Gram*	*Units/gram*
1. Soybean meal							
No. 1	19.6	2.99	2.37	607	0.5	0.01	34,700
Blank	64.0	0.62				0.01	
No. 2	19.3	3.03	2.41	617	0.5	0.01	35,200
Blank	64.0	0.62				0.01	
2. Green beans							
No. 1(a)	14.4	3.67	2.91	746	7	0.5	72.0
No. 1(b)	28.3	2.20	1.44	369	4	0.5	
Blank	59.3	0.76				0.5	

A. DETERMINATION OF FERRIC THIOCYANATE IN ALCOHOL-WATER SOLUTION. Ten cubic centimeters of the above mixture are transferred to the colorimeter cell. Exactly 15 minutes after the addition of the ferrous salt (15 minutes was found necessary for complete decomposition of the fat peroxide), 10 cc. of ethyl alcohol are added to the contents of the cell. This removes the turbidity produced by the presence of the fat. One cubic centimeter of 20 per cent ammonium thiocyanate is added and the colorimeter cell (a large test tube) is shaken. The tube is placed in the colorimeter and the galvanometer scale reading is recorded. [Wratten filter No. 65 (green) was used in the colorimeter.] Atmospheric oxidation of the ferrous iron produces a slow drift in the scale reading if the solution stands for any length of time.

B. DETERMINATION OF FERRIC THIOCYANATE IN ISOAMYL ALCOHOL. Twenty cubic centimeters of the oxidized fatty acid solution are transferred by means of a pipet to a 100-cc. test tube, and exactly 5 cc. of isoamyl alcohol are added. Then 5 cc. of a solution of 20 per cent ammonium thiocyanate are added, and the test tube is stoppered and tipped slowly upside down about six times. This gives complete extraction of the color without producing emulsions or turbidity. The layers are allowed to separate, 5 cc. of isoamyl alcohol are added and uniformly mixed by gentle swirling of the tube, and 5 cc. of the red isoamyl alcohol layer are pipetted into a 25-cc. volumetric flask. The flask is filled to the mark with isoamyl alcohol. The color may be measured in a photoelectric colorimeter, as in procedure A, or in a Duboscq colorimeter.

In either procedure the ferric iron content of the solution is determined in micrograms per cubic centimeter by reference to a standard curve, or by comparison with the color formed by a standard iron solution. From these data the total ferric iron in the reaction mixture may be calculated. A simple straight-line graph is convenient for this calculation.

With either method, a blank determination must be used to compensate for the iron in the reagents and for atmospheric oxidation during the manipulation. The blank determination is carried out exactly as above, except that the enzyme extract is added after the solution has been acidified, so that there is no enzyme action. The ferric iron value obtained is subtracted from the value for the active reaction mixture.

Procedure A is rapid and economical, but requires the use of a photoelectric colorimeter, since the red color in water and ethyl alcohol is not sufficiently stable for use in a visual colorimeter. Procedure B is designed for application of the method when a photoelectric instrument is not available.

Application of the Method

On the basis of this analytical technique, a unit of lipoxidase was defined as the activity which, in the presence of 5 mg. of linoleic acid in the described mixture, catalyzes the reaction of 1 microgram of oxygen in 1 minute at 25° C. at pH 7.

Using aqueous suspensions of the pulverized materials as enzyme extracts, the lipoxidase content of various enzyme sources was compared. Since in the early stages the amount of peroxidation was not directly proportional to the time of reaction, it was necessary to establish the configuration of the reaction curve in each case. The enzyme concentration was calculated from a time interval lying on the straight-line portion of the curve. A collection of illustrative data is presented in Table I.

In Table I, the galvanometer readings indicate the amount of light transmitted by the ferric thiocyanate solution. The calibration curve formed by plotting the galvanometer readings against the ferric thiocyanate concentration is logarithmic in form. Hence, at high color concentrations the sensitivity of the method decreases, the useful limit of concentration being about 4 micrograms of ferric iron per cc.

It is obvious from Table I, that the use of a short reaction time and a diluted enzyme extract multiplies the error when calculating the lipoxidase concentration, although in such

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TABLE II. QUANTITATIVE COMPARISON OF LIPOXIDASE SOURCES

Enzyme Source	Lipoxidase Unitage per Gram Fresh weight	Dry weight
Soybean meal[a]	...	35,000
Peas (green Telephone)	1,400	6,220
Potatoes (fresh Irish Cobbler)	378	2,150
Wheat germ	...	810
Green string beans	71	790
Alfalfa, freshly cut	26	90
Asparagus (Green Jumbo)	7.2	74

[a] Dry Seneca soybeans, ground and thoroughly extracted with petroleum ether at 25° C.

cases the reaction curve can be considered to be a straight line, which simplifies the determination. In the second example presented, the enzyme concentration was calculated from a 3-minute interval lying on the straight-line portion of the curve formed by plotting peroxidation against time.

Table II presents the results of the investigation.

Summary and Conclusions

A rapid colorimetric method for the determination of the activity of the enzyme lipoxidase is based upon the fact that the resulting fat peroxide oxidizes ferrous iron. The ferric ion formed is determined as the colored thiocyanate complex by means of a photoelectric colorimeter.

The lipoxidase content of several vegetables was compared by means of this method.

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Separation and Determination of Protein Sulfur, Sulfide Sulfur, and Other Sulfur

In Sodium Sulfide Dispersions of Keratins

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DURING a study by the junior author of the dispersion of certain keratins in solutions of sodium sulfide, it became desirable to determine separately the protein sulfur, sulfide sulfur, and, as a third fraction, other sulfur compounds. A review of the literature failed to provide a method suitable for such a separation. Theis and Ricker (*9*) studied the inorganic sulfur compounds produced during prolonged action of alkaline sulfide solutions on cowhide, but they reported no work on the protein sulfur.

Several methods for obtaining the desired separation were tried, including treatment of the dispersion with mineral acids, with lead acetate, and with trichloroacetic acid.

Treatment of the dispersion with mineral acids would release hydrogen sulfide from sulfides present, but this treatment would also release sulfur dioxide from sulfites and bisulfites and, if the acid were sufficiently concentrated, sulfur from thiosulfates. The acid concentration required to precipitate the protein was sufficiently high to release sulfur dioxide from sulfites and precipitate sulfur from thiosulfates.

Precipitation of the dispersion with normal lead acetate, filtering, and washing with hot ammonium acetate and then with hot water separated the other sulfur compounds from the protein and lead sulfide formed but provided no means of separating the two latter groups.

The protein dispersion is colloidal, and mineral salts are effective in flocculating the disperse phase. As aluminum salts are very effective in flocculation (*6*) a water suspension of basic aluminum acetate was found to precipitate the protein present and also to liberate hydrogen sulfide from the sodium sulfide present. The use of basic aluminum acetate was also found to be applicable to the separation of sulfides from sulfites, thiosulfates, and sulfates.

Three dispersions were analyzed by the method discussed in this paper.

The protein materials used were chicken feathers, hog hair, and wool. The sulfur contents of these materials, based upon their air-dry weight, were: chicken feathers, 2.3 per cent; hog hair, 5.6 per cent; and wool, 3.6 per cent. The keratin dispersions were prepared by a modification of the method described by Goddard and Michaelis (*4*). These dispersions contained about 7 per cent of the protein material in 0.1 *M* sodium sulfide. Total sulfur was determined in the protein materials by an alkaline permanganate fusion (*8*). The official magnesium nitrate ignition (*2*) and the alkaline permanganate fusion (*8*) were used on the precipitated proteins, and the two methods gave the same results. Magnesium nitrate did not wet the chicken feathers, and so was not used for the determination of total sulfur in this material.

The basic aluminum acetate method was also tried on solutions of sodium sulfide, both by itself and mixed with some other sulfur salts, to determine whether it would be successful in separating sulfide sulfur from other sulfur compounds. Also, sodium sulfide and other sulfur salts were added to the feather dispersion and the sulfide sulfur was separated by the aluminum acetate.

Reagents

Basic aluminum acetate, suspension of 5 grams in 100 ml. of water.

Lead acetate, basic, dry powder, a 1 per cent solution, as for sugar analysis by the Horne method.

Bromine–hydrobromic acid, equal volumes of 48 per cent hydrobromic acid and bromine.

Magnesium nitrate, dissolve 150 grams of magnesium oxide in nitric acid (1 + 1), avoiding an excess of acid. Add a little magnesium oxide in excess, filter from excess of magnesium oxide, iron oxide, etc., and dilute to 1 liter (*1*).

Potassium permanganate, a saturated solution.

Sodium hydroxide, a 15 per cent solution; also solid.

Nitrogen, compressed.

Hydrochloric acid, concentrated and diluted 1 to 1 with water.

Sodium carbonate, solid, anhydrous.

Methyl orange, water solution, 0.5 gram per liter.

Barium chloride, a 10 per cent solution.

Carbon disulfide, analytical reagent.

N-Octadecyl alcohol, a saturated solution in 95 per cent ethyl alcohol.

Ammonium hydroxide, concentrated.

Procedure

A 2-ml. sample (weighed) of the keratin dispersion was washed into a flat-bottomed vial (*D*, Figure 1) with the minimum amount of water necessary and 5 ml. of *n*-octadecyl alcohol reagent were added to minimize foaming. Basic aluminum acetate suspension was added, the mixture was heated, and the hydrogen sulfide evolved was swept with nitrogen into a basic lead acetate solution. This reaction was carried out in the apparatus shown in Figure 1.

Two milliliters of the basic aluminum acetate were drawn into the pointed tube, *C*, a clamp at *A* was tightened and another at *B* loosened, the T-tube was connected, and *C* was lowered into the solution in vial *D*. Nitrogen was then turned on so that 3 or 4 bubbles a second passed through the water in bottle *H*. Connections were completed with test tubes *F* and *G*; vial *D* was lowered into boiling water in the 400-ml. Griffin beaker, *E*; the clamp at *A* was loosened and that at *B* tightened, whereupon the stream of nitrogen drove the basic aluminum acetate into the dispersion, precipitating the protein and evolving hydrogen sulfide from sulfides present. The hydrogen sulfide was carried into the basic lead acetate solution in *F*, precipitating lead sulfide. Tube *G* also contained basic lead acetate and was used only as a precaution. All the hydrogen sulfide was driven off in 5 to 10 minutes and further heating was avoided, since it caused the protein to stick to vial *D*. The stopper containing tube *C* was removed from *D* while the nitrogen was still bubbling through it to prevent protein material from collecting inside the tube.

The protein was broken up with a rubber-tipped stirring rod, transferred to filter paper in a Büchner funnel, and washed thoroughly with hot water. The final volume of the filtrate was usually about 250 to 300 ml. The protein was then ignited with about 15 ml. of magnesium nitrate solution or with 10 ml. of permanganate solution plus 1 ml. of 15 per cent sodium hydroxide.

The alkaline permanganate fusion was made at 600° C. instead of at 500° C. as in the original method (*8*), but after fusion the mixture was treated as directed by Pollock and Partansky (*8*). The magnesium nitrate ignition mixture was analyzed by the A. O. A. C. method (*2*). After the ignition mixture was dissolved, the solution was made just acid to methyl orange by the proper use of ammonium hydroxide and hydrochloric acid. About 0.5 ml. of hydrochloric acid per 100 ml. of solution was then added and the solution was heated to boiling. Two milliliters of the barium chloride solution were then slowly added from a pipet, with constant stirring of the solution. The solutions were kept at about 40° C. overnight, filtered through weighed Gooch crucibles, and the precipitated barium sulfate was washed with hot water. The barium sulfate was then ignited at a cherry red heat in a muffle furnace (about 650° C.) to constant weight, cooled for an hour in a desiccator, and weighed.

The filtrate from the precipitated protein contained the other sulfur compounds. To it were added about 5 grams of sodium hydroxide and 5 ml. of bromine water. The solution was kept alkaline (*3*). It was heated on a steam bath for an hour, acidified with hydrochloric acid (1 + 1), and boiled to expel bromine, and the sulfur was determined as before.

The sulfide sulfur was determined by dissolving the lead sulfide in tube *F* (Figure 1) in 3 ml. of bromine–hydrobromic acid mixture, heating gently until all bromine was driven off, then cooling and neutralizing with sodium carbonate. Tall-form beakers were used to avoid loss by effervescence. An excess of about 1 gram of sodium carbonate was added and the solution was boiled for about 15 minutes. Lead carbonate was precipitated and sodium sulfate remained in solution. The lead carbonate was filtered off and freed from sodium sulfate by washing. The filtrate was then made slightly acid with hydrochloric acid (1 + 1) and the sulfur was determined as before.

The amount of basic aluminum acetate necessary to precipitate the protein and drive off the hydrogen sulfide was determined by treating the dispersion with measured quantities of basic aluminum acetate, boiling until the hydrogen sulfide was all evolved, and testing the solution with a drop of 10 per cent normal acetate. The time necessary to remove the hydrogen sulfide was also determined by testing at intervals with lead acetate paper. Care was taken to have all rubber and glass connections perfectly dry before the operation was started, so that no hydrogen sulfide would be dissolved in the water present.

Preliminary determinations of the sulfur content of methionine and cystine were made by the official magnesium nitrate method and the alkaline permanganate fusion method. The results, given in Table I, show that a fusion temperature of 600° C. must be used with the alkaline permanganate fusion method to obtain the true amount of sulfur in methionine. The results obtained by this method compared favorably with those obtained by the magnesium nitrate ignition.

Results and Discussion

Three sodium sulfide dispersions of keratins were analyzed for total sulfur by fusion and the three fractions of sulfur compounds separated from each dispersion by the use of basic aluminum acetate were also analyzed for sulfur. The analyses were made in duplicate or triplicate on freshly prepared dispersions. Analyses were also made of dispersions

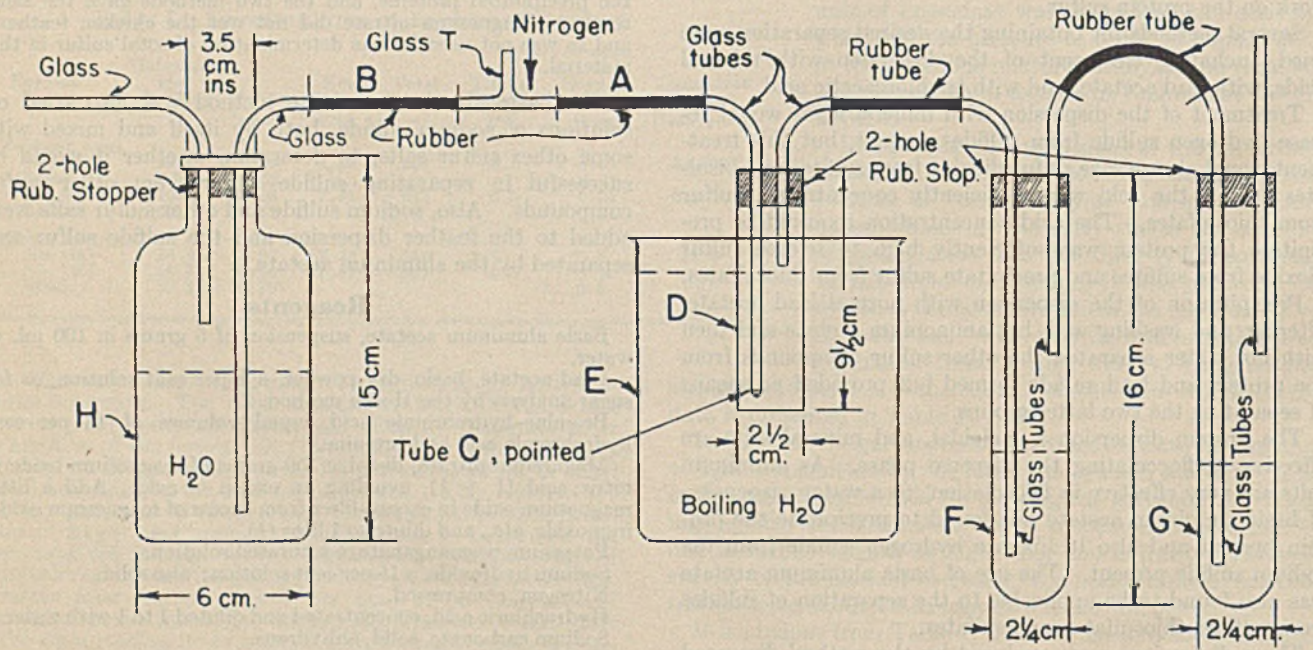

FIGURE 1. DIAGRAM OF APPARATUS

TABLE I. DETERMINATION OF SULFUR

(Comparison of magnesium nitrate method and alkaline permanganate method)

Material	Sulfur Obtained: Magnesium nitrate method	Alkaline permanganate method 500° C.	Alkaline permanganate method 600° C.	Sulfur theoretical
	%	%	%	%
d,l-Methionine	21.52	16.97	21.56	21.47
	21.55	16.98	21.53	...
l-Cystine	26.20	...	26.40	26.63
	26.36	...	26.44	...

TABLE II. BASIC ALUMINUM ACETATE SEPARATION OF SULFUR IN SODIUM SULFIDE DISPERSIONS OF KERATINS

	Protein Sulfur	Sulfide Sulfur	Other Sulfur	Sum	Total Sulfur by Fusion Method
	Mg./g.	*Mg./g.*	*Mg./g.*	*Mg./g.*	*Mg./g.*
Feather dispersion, freshly prepared	2.3	2.1	1.1	5.5	5.3
	2.4	1.9	1.1	5.4	5.5
Feather dispersion, stood 1 month	1.8	None	3.6	5.4	...
Hog hair dispersion, freshly prepared	4.1	1.5	0.7	6.3	6.1
	4.4	1.3	0.7	6.4	6.1
	4.1	1.4	0.6	6.1	...
Hog hair dispersion, stood 15 days	4.2	None	2.2	6.4	...
	4.1	None	2.2	6.3	...
Wool dispersion, freshly prepared	3.2	1.5	0.6	5.3	5.6
	3.4	1.7	0.5	5.6	5.5

that had stood in the laboratory for some time. The results of these analyses are given in Table II.

To test for the possible formation of elemental sulfur in the protein fraction upon standing, the protein was separated from duplicate samples of a hog hair dispersion that had stood in the laboratory for 15 days. One protein fraction was analyzed as usual; the other was washed twice with boiling carbon disulfide, after which it was dried and analyzed for sulfur. The results were nearly identical, showing that no sulfur had been removed from the second protein fraction; moreover, the residue from the carbon disulfide used in washing contained no sulfur.

The pH of a freshly prepared feather dispersion was 12.0. One milliliter of this material was diluted with 5 ml. of water (resulting pH 10.6) and boiled with 2 ml. of basic aluminum acetate (pH 4.5) until all the hydrogen sulfide was driven off. The solution was then cooled and again tested for pH, which was found to be 4.5.

Mixtures of the feather dispersion and various sulfur compounds were analyzed by the basic aluminum acetate method as described. Recovery of the added sulfur was complete and the amounts of protein sulfur and sulfide sulfur in the dispersion were not affected. When sodium sulfide was added to the dispersion, the protein sulfur remained unchanged, the sulfide sulfur increased by the amount present in the sodium sulfide, and the other sulfur fraction also increased by the amount present in the added salt. When solutions of sodium sulfite, sodium thiosulfate, and potassium sulfate were added to the protein dispersion, analyses showed that the protein and sulfide sulfur of the dispersion remained unchanged, the sulfur of the added salt being included in the remaining sulfur of the dispersion. Sodium bisulfite, when added to the dispersion, did not behave like the other added salts; an increase was observed in both the protein and sulfide sulfur fractions. Results of the analyses of mixtures of the protein dispersions with added sulfur compounds are given in Table III.

The sulfur distribution of a keratin dispersion in sodium sulfide solution is probably the result of an equilibrium between several reactions involving the inorganic sulfide ion and the disulfide sulfur of the protein. Part of the original sulfide is oxidized to disulfide or to a polysulfide during reduction of the cystine residues of the keratin (*4*). Another reaction that would also result in a decrease of the sulfide-ion concentration is that described by Nicolet and Shinn (*7*). In this reaction a part of the original sulfide sulfur is incorporated into the protein molecule by addition to the double bonds formed by the action of alkali on the hydroxy amino acid residues of the original protein. On the other hand, it is possible that the final sulfide sulfur might include that part of the cystine sulfur which appears as inorganic sulfide during the conversion of cystine to lanthionine—a conversion which, as Horn and Jones (*5*) have shown, occurs upon treatment of proteins with sodium sulfide.

It is evident from the foregoing discussion that no conclusion can be drawn from the data in Table II as to the sources of the sulfur in the three fractions. Indeed, this problem lies outside the scope of the present paper. However, it is hoped that the method of separation and determination of the sulfur fractions described here may be a useful tool (with cystine analyses, for example) in studies of the reactions involved in the dispersion of keratins by sodium sulfide and of the action of alkali on the sulfur of proteins.

TABLE III. BASIC ALUMINUM ACETATE SEPARATION OF SULFUR IN KERATIN DISPERSIONS PLUS ADDED SULFUR COMPOUNDS

	Protein Sulfur	Sulfide Sulfur	Other Sulfur	Sum	Total Sulfur[a]
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Protein dispersion (1 gram)	2.3	2.1	1.1	5.5	5.4
	2.4	1.9	1.1	5.4	5.5
Sodium sulfide (1 ml.)	None	2.2	1.4	3.6	3.4
	None	2.3	1.4	3.7	3.6
Protein dispersion (1 gram) + sodium sulfide (1 ml.)	2.3	4.2	2.4	8.9	9.4
	2.4	4.1	2.5	9.0	9.2
Protein dispersion (1 gram) + sodium thiosulfate (1 ml.)	2.5	1.9	7.7	12.1	11.9
	2.3	2.0	7.9	12.2	12.2
Protein dispersion (1 gram) + sodium sulfite (1 ml.)	2.4	1.9	2.7	7.0	6.9
	2.3	2.0	2.6	6.9	6.9
Protein dispersion (1 gram) + potassium sulfate (1 ml.)	2.5	2.0	4.4	8.9	8.6
	2.4	1.9	4.5	8.8	8.7
Protein dispersion (1 gram) + sodium bisulfite (1 ml.)	2.8	2.9	1.2	6.9	6.7
	2.9	2.6	1.1	6.6	7.0

[a] Alkaline permanganate fusion method.

Summary of Results

The sulfur in sodium sulfide dispersions of keratins may be separated into three parts: protein sulfur, sulfide sulfur, and other sulfur compounds by means of basic aluminum acetate.

Sulfides may be separated from sulfites, thiosulfates, and sulfates, but not from bisulfites, by treatment with basic aluminum acetate.

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Determination of Pure Carotene in Plant Tissue

A Rapid Chromatographic Method

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A RESEARCH project at this laboratory involved the accurate determination of the carotene content of many samples of fresh and dehydrated vegetable products. (Carotene referred to in this paper includes both alpha- and beta-carotene, in most cases approximately 10 per cent alpha and 90 per cent beta.) The methods commonly used at present for the determination of carotene in plant materials depend upon the phasic separation of carotene from interfering pigments. The carotene is extracted from the plant material with solvents, such as alcohol or acetone, and then usually removed from the original extractant with petroleum ether. A number of workers (*1*, *3*, *4*, *9*) have shown, however, that the petroleum ether phase obtained from dehydrated and stored plant materials or silage may still contain, after final purification, noncarotene chromogens, which are estimated as carotene.

Several methods (*1*, *2*, *4*) have been published in which the noncarotene chromogens are removed by contact adsorption or by passage through a Tswett column. In these procedures it is often necessary to activate the adsorbent and to carry out preliminary phasic separation with petroleum ether from the original solvent.

A procedure has been devised at this laboratory for the determination of carotene in dehydrated plant products with an adsorbent that requires no special activation and no phasic separation. The steps in brief consist of (1) extraction with a mixture of 30 per cent acetone and 70 per cent Skellysolve B, (2) removal of most of the acetone by evaporation, (3) separation of the carotene from interfering pigments by passage through an adsorption column of activated magnesium oxide, and (4) washing with 3 to 5 per cent acetone in Skellysolve B. The adsorbent is a modified mixture of Micron Brand activated magnesium oxide No. 2641 and Hyflo Super-Cel, first introduced by Strain (*8*) for the chromatographic separation of various carotenoid pigments.

Procedure

EXTRACTION OF CAROTENE. Dehydrated plant material (1.00 gram), ground to 40-mesh or smaller, with a carotene range of 50 to 500 micrograms per gram is used for the determination. One-half or 2-gram samples should be taken if the carotene content deviates from this range. The apparatus used at this laboratory is either a 300-ml. standard taper Erlenmeyer flask with a condenser and heated on a hot plate, or a Soxhlet apparatus with a 500-ml. Soxhlet flask. The material is refluxed for half an hour with 100 ml. of a mixture of 30 per cent acetone and 70 per cent Skellysolve B in the first apparatus, or for 1 hour with 200 ml. if the Soxhlet apparatus is used, operating at a rapid siphon rate. If the former apparatus is used, the sample is filtered on a sintered-glass funnel (coarse porosity) into a 500-ml. filter flask and washed with approximately 25 ml. of the Skellysolve three or four times. The filter flask, or the Soxhlet flask, containing the extract is placed directly in a steam bath and evaporated to 25 to 50 ml. Under no circumstance should all the solvent be evaporated, since this would destroy some of the carotene. This extraction procedure removes all the carotene but not all the chlorophyll.

SEPARATION OF CAROTENE FROM OTHER PIGMENTS BY CHROMATOGRAPHIC ADSORPTION. (This laboratory is at present using similar principles in large-scale preparation of carotene.) The adsorbent used is composed of three parts of Hyflo Super-Cel and one part of Micron Brand magnesium oxide, a specially activated product readily obtained on the commercial market at low cost. A 453-gram (1-pound) batch of adsorbent is conveniently prepared by spreading the Super-Cel and magnesium oxide on a large sheet of paper and mixing by turning over the heap approximately ten times, or rotating in a ball mill with the balls omitted.

The adsorption tube is made from a test tube approximately 23 × 200 mm. with a piece of glass tubing attached to the bottom. A plug of cotton or glass wool is put in the bottom, and the adsorption tube is connected to a suction flask or a large vacuum desiccator or suction bell jar. If the two latter devices are used, the receiver may be a 100-ml. or 250-ml. volumetric flask, thus avoiding later transference of the solution to the volumetric flask. With the full vacuum of a water pump, the adsorbent is added until the height of the column is about two-thirds to three-fourths the height of the adsorption tube. All the adsorbent is firmly pressed with a plunger consisting of a glass rod and properly fitting cork, and the column is washed with approximately 50-ml. of Skellysolve, which is then discarded. The evaporated extract is poured on the wet adsorbent column, which removes all the pigments. The flask and column are washed with a mixture of 3 to 5 per cent acetone in Skellysolve B until the solvent comes through colorless. The washings may be continuous or intermittent. Usually a total of 100 ml. is necessary. Full suction must be used with all the operations.

The chlorophyll and xanthophyll are held firmly at the top of the column, as are most of the other noncarotene pigments. The carotene is washed through the column by the acetone-Skellysolve mixture. The acetone is necessary because pure Skellysolve elutes the carotene from the column too slowly to be of value in routine analysis. Occasionally a small amount of noncarotene chromogen may be slowly washed down the column. The carotene is eluted much more rapidly, however, and no interference results, particularly if the final washings are pure Skellysolve B. In most cases the adsorbent may be used a number of times before it is discarded. The whole procedure of adsorption and elution, usually run in duplicate, takes from 5 to 10 minutes.

DETERMINATION OF CAROTENE. The carotene solution is made to volume and determined in any of the usual ways. In this laboratory a Lumetron photoelectric colorimeter provided with an H-4 mercury arc light and with two Corning No. 511 filters plus a neutral gray filter or two Corning No. 511 filters plus a Corning Noviol N 038 filter were used. Since the carotene solution being estimated was relatively pure, both filter combinations gave the same results, but the latter filter set, transmitting at 440 mμ, is intrinsically more accurate. A standard calibration curve was made from S. M. A. carotene that was 90 per cent beta and 10 per cent alpha, purified according to the method of Fraps and Kemmerer (*1*). All the carotene in the samples was estimated as beta-carotene, although a small but variable fraction was alpha-carotene.

Extraction of Fresh Samples

A procedure developed by Moore and Ely (*5*) in which the Waring Blendor is used to extract carotene from fresh plant samples by means of a foaming mixture of alcohol and petroleum ether was tested, and with some slight modifications proved to be excellent.

A 10-gram sample of finely minced plant material is extracted with 150 ml. of 95 per cent ethyl alcohol and 75 ml. of Skellysolve B in a Waring Blendor for 5 to 10 minutes. More alcohol is added if the mixture does not foam. The authors found it convenient to regulate the speed of the blender with a voltage controller. The extract and finely divided plant material are transferred to a fritted-glass filter by means of a piece of wide-bore glass tubing connected to the filter with a rubber stopper. The filter is attached to a suction flask. The container of the blender is alternately washed with alcohol and Skellysolve until the filtrate running into the suction flask is colorless. It was found advisable to remove the alcohol and extracted water from the Skellysolve in a separatory funnel. One hundred milliliters of water containing about 5 grams of sodium sulfate are added to the alcohol-Skellysolve mixture, and the lower aqueous alcohol solution is drawn off. The aqueous solution is extracted three times with 30 ml. of Skellysolve. The sodium sulfate aids in clearing troublesome emulsions, which may occur from time to

Table I. Recovery of Pure Carotene after Passage through Magnesium Oxide Adsorbent

Stock solution	Extinction Stock solution adsorbed	Recovered %
0.500	0.510[a]	102.0
0.534	0.532	99.6
0.260	0.260	100.0
0.260	0.255	98.1
		Av. 99.9

[a] Stock solution concentrated in vacuo prior to adsorption.

time. Fifty milliliters of water are poured through the combined petroleum ether fractions, the water is drawn off, and the petroleum ether is concentrated to approximately 25 ml. The magnesium oxide is stable to small amounts of alcohol, so that the prolonged extraction of the petroleum ether with water to remove alcohol, as described by Moore (*4*), is unnecessary. A few grams of anhydrous sodium sulfate are added to the Skellysolve to remove moisture, and a small layer of sulfate is placed above the adsorbent as a precautionary measure. The rest of the procedure is the same as that described for dehydrated material.

Discussion and Results

The various steps in the procedures were carefully checked.

Carotene Recovery. A purified sample of 90 per cent beta and 10 per cent alpha-carotene was used for carotene recovery tests. An aliquot of a stock solution was made to a 100-ml. volume and read in the colorimeter. A similar aliquot was washed through the adsorption column with 3 to 5 per cent acetone in Skellysolve B, made to volume, and read. In other cases the aliquot was made to 100 ml., concentrated in vacuo, and washed through the adsorbent.

The results are shown in Table I.

The recovery of carotene added to samples of dehydrated, stored pea and lima bean vines that had been shown to contain considerable noncarotene chromogens is shown in Table II. These results show that by the extraction and adsorption procedure outlined quantitative recovery of carotene may be obtained both with pure carotene (Table I) and with carotene added to plant samples (Table II).

Effect of Evaporation on Carotene. Data on the effect of vacuum versus steam concentration of the petroleum ether extracts are given in Table III. Carotene is not affected by evaporation on a steam bath under the experimental conditions.

Comparison of the Adsorption Method with Phasic Procedures. The determination of carotene in dehydrated and fresh or frozen plant materials by the adsorption method was compared with the widely used Peterson-Hughes-Freeman (*7*) technique as modified by Peterson (*6*). After the carotene was determined in the Skellysolve extract obtained by the Peterson-Hughes-Freeman method, an aliquot of the same solution was passed through the magnesium oxide absorbent column. Other samples were analyzed directly by the method described here. In this way a comparison of the extraction and determination of carotene by the new technique could be obtained, since the Peterson-Hughes-Freeman technique and its modifications give excellent extraction of carotene. The results shown in Table IV were obtained on samples dehydrated and stored for different periods of time, on others dehydrated and analyzed immediately, and on fresh and frozen samples.

The results in Table IV are typical of those obtained on a large number of samples. It is apparent that the phasic method records as carotene a considerable amount of noncarotene material, since on passing the extracts through the magnesium oxide column lower values are always obtained.

Table II. Recovery of Carotene Added to Dehydrated Plant Samples

Carotene in Sample	Carotene Added	Carotene Theoretical	Carotene Found	Recovered
γ	γ	γ	γ	%
72.6	114.3	186.9	190.0	101.5
8.6	114.3	122.9	122.8	100.0
37.2	114.3	151.5	152.2	100.5
62.8	114.3	177.1	171.5	96.8
77.9	114.3	192.2	190.0	98.8
				Av. 99.5

Table III. Effect of Evaporation on Carotene

Dehydrated Sample	Carotene Vacuum	Steam
	Micrograms per gram	
Savoy cabbage leaves	280.0	282.0
Broccoli leaves	281.0	270.0
Carrots (vacuum-dried)	486.0	486.0
Carrots (air-dried)	70.4	71.4

Table IV. Carotene Content of Dehydrated and Fresh Vegetable Products

(Determined by the Peterson-Hughes-Freeman technique and by the adsorption procedure)

	Carotene Found		
	PHF procedure	PHF procedure followed by adsorption	Adsorption procedure
	Micrograms per gram		
Dehydrated samples			
Pea vines	73.6	47.0	46.2
Broccoli leaves and petiole	259.0	186.0	192.0
Hubbard squash rind	68.7	39.6	40.0
Pepper	45.7	20.4	20.4
Lima bean leaves	209.0	164.0	170.0
Lima bean vines, laboratory dried	76.0	61.3	59.3
Lima bean vines, commercially dried	53.2	27.8	26.0
Asparagus tops	379.0	334.0	310.0
Spinach	878.0	828.0	810.0
Fresh or frozen samples			
Lima bean silage	36.8	21.2	21.2
Frozen savoy cabbage leaves[a]	372.0	293.0	292.0
Frozen lima bean leaves[a]	363.0	344.0	348.0
Frozen carrots and peas	94.0	...	95.5
Fresh spinach	87.8	76.5	74.3

[a] Results expressed on dry-weight basis.

These lower values agree very well with those obtained by the present method. The adsorption procedure not only removes chlorophyll and xanthophyll but also the less common pigments, such as lycopene and the noncarotene pigments of peppers, squash, and pumpkin. Samples that had been dehydrated and stored showed a considerably greater proportion of noncarotene pigments than dehydrated samples analyzed immediately. Samples stored for 3 to 6 months at room temperature lost large amounts of carotene, some undoubtedly being converted into noncarotene pigments, which are estimated as carotene by phasic procedures. Moore (*4*) and Fraps and Kemmerer (*1*) have presented similar results. The adsorption procedure also removes considerable noncarotene impurities from silage. On the whole the results of the phasic procedures show better agreement with those of the adsorption technique when fresh and frozen vegetable materials are used than on dried or ensiled samples.

A comparison of the values obtained by the magnesium oxide adsorbent with those obtained by the Baker's C. P. analyzed dicalcium phosphate adsorbent described by Moore (*4*) indicates that the adsorbents would give identical results. In many cases, however, it was necessary to readsorb the eluate that had passed through the dicalcium phosphate in order to remove small amounts of chlorophyll. In addition, the dicalcium phosphate at times may have to be specially activated in the laboratory. It is very susceptible to traces of polar solvents and much more expensive than the infusorial earth–magnesium oxide mixture, so that it is not so well adapted to routine procedures.

Duplicates run by the adsorption method agree within 3 to 5 per cent. It is difficult to estimate the accuracy of the determination, since a small and variable amount of alpha-carotene and possibly, with some materials, other carotene isomers are estimated as beta-carotene. Carotene-recovery tests and comparison with other extraction procedures indicate, however, that the adsorption procedure is considerably more accurate than phasic procedures in determining the carotene content of dehydrated and stored feeds or silage and the recovery of carotene by this method is of a high order. The authors have observed that the adsorbent will qualitatively separate alpha- and beta-carotene if the column is washed with pure Skellysolve B or Skellysolve B and 1 per cent or less acetone, and that carotene from yellow corn may easily be separated from cryptoxanthin by this procedure.

Summary

A rapid, reproducible method for the determination of carotene in plant materials, either dehydrated or fresh, has been described in detail. The method for dehydrated products consists essentially of extraction of carotene with a mixture of 30 per cent acetone and 70 per cent Skellysolve B, and subsequent separation of the carotene (alpha and beta) from interfering pigments on a column of three parts of Hyflo Super-Cel and one part of Micron Brand activated magnesium oxide. This adsorbent requires no special laboratory activation and does not destroy carotene. For dehydrated materials, phasic operations are completely eliminated, thus making the method much more rapid than any of the published phasic procedures. Fresh material is extracted by modification of the Moore and Ely method in which the Waring Blendor is used. A comparison of the results obtained by the method described with those obtained by the Peterson-Hughes-Freeman method indicates that considerable amounts of noncarotene pigments are estimated by procedures based on the phasic separation of carotene from noncarotene pigments.

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Studies in Filter Photometry

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THE single barrier layer photocell is one of the most widely used instruments of this nature, and has been subject to the most varied manner of empirical calibration. There is a definite need for improvement, particularly in respect to the exactness and fundamental nature of the measurements made and being reported with these instruments. Müller (*3*) has reviewed the literature of photoelectric photometers comprehensively; hence there is no need of considering this aspect further.

The most important use of filter photometers is in colorimetric analysis. When the solution obeys Beer's law, the relationship between log transmittance and concentration is linear. The need for instrument calibration in transmittance or its logarithm is obvious. Sheard and States (*6*) have summarized the situation existing with the use of barrier layer photocell photometers when they state that failure to obey Beer's law has been ascribed more frequently to the photocell than to the lack of monochromatic illumination. Experimental work was undertaken to determine the effect of the wave band of spectral illumination on the type of analytical calibration curve obtained. The colorimetric determination of silica by means of the silicomolybdic acid reaction was used as a test method and is particularly interesting because the absorption occurs in a spectral region of minimum sensitivity.

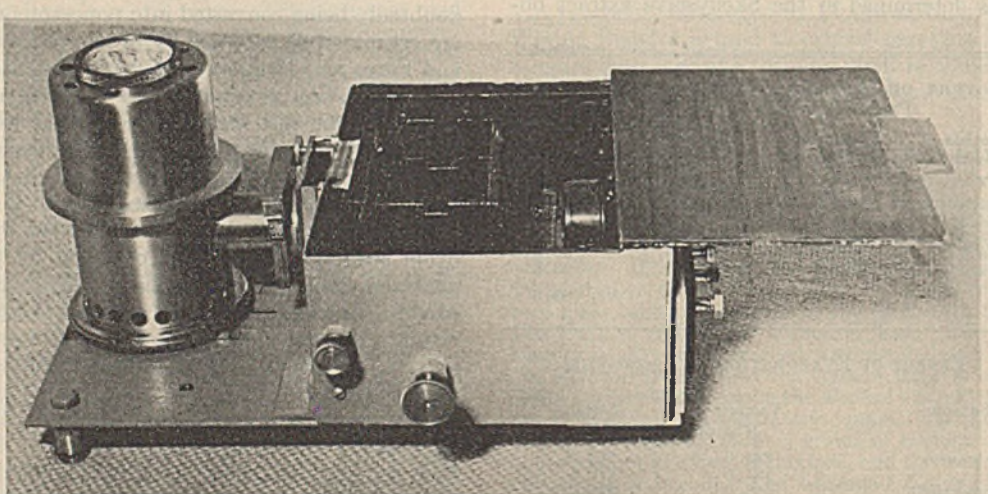

FIGURE 1. FILTER PHOTOMETER

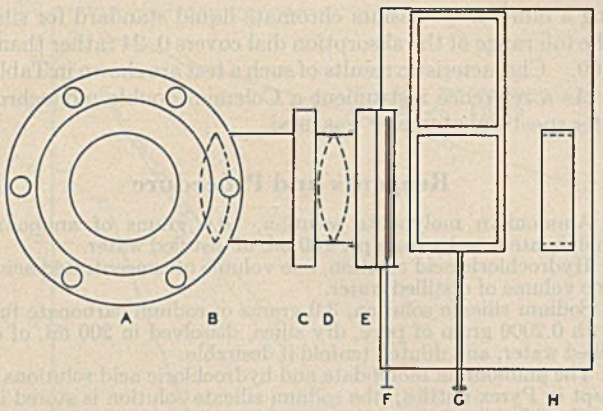

FIGURE 2. FILTER PHOTOMETER

A. Light	*E.* Filter holder
B. Lens	*F.* Shutter
C. Heat-absorbing filter holder	*G.* Absorption cell holder
D. Lens	*H.* Barrier layer photocell

Filter Photometer

The photometer consists of lamp and ventilated lamp housing to which is attached a collimating tube containing a lens for producing parallel rays of light, a holder for a heat-absorbing filter, and another lens for producing a very slightly divergent beam of light. A light-tight box with hinged cover contains a shutter and an external handle which moves it in and out of the path of light, a holder for two absorption vessels which may alternately be moved in and out of the path of the light by an external handle, and a barrier layer cell. On the outside of the box is the holder for filters. An air gap is provided between the two separate portions of the photometer but stray light is excluded by overlapping collimating tubes. The apparatus is pictured in Figure 1.

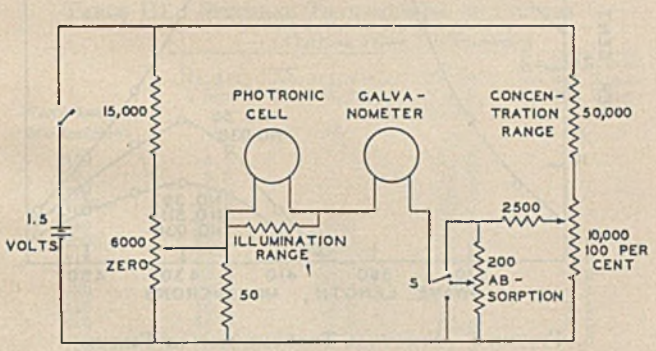

FIGURE 3. FILTER PHOTOMETER CIRCUIT

A schematic diagram of the filter photometer is represented in Figure 2 and the electrical circuit for obtaining potentiometric photocurrent balance is presented in Figure 3. The light source is a 32- or 50-candlepower, 6- to 8-volt lamp, the lenses are of the simple convex type, the heat-absorbing filter is Corning No. 397, the absorption cells are 50 × 50 × 50 mm. and 50 × 50 × 10 mm., the barrier layer cell is a Weston Model No. 594, Type 1 or Type 2, and the filters are of Corning glass. The measurements are actually made in terms of per cent absorption from which per cent transmittance is easily computed. Since the position of the lamp holder is adjustable, the light source can be easily changed. In addition to the lamps mentioned above a Westinghouse 85-watt mercury vapor lamp was used and a 110-volt, 100-watt prefocus lamp. A heavy-duty storage battery, trickle charger, G. M. galvanometer No. 2564-C, 1.5-volt dry cell, General Radio Co. variable resistors, and International Resistance Co. fixed resistors complete the list of apparatus.

In order to secure photocurrent from the photocell proportional to the light intensity it is necessary to use sufficiently low values of external circuit resistance. However, it is also true that when the light intensity and photocurrent are low, the linear response of the photocell is less affected by an increased value of the external resistance. In fact, if the product of external resistance times the current delivered—that is, the terminal potential of the barrier layer cell—is assigned an upper limit by the conditions of the experiment, then there exists assurance that the non-linearity will be below some fixed value. This is obtained in the circuit employed, using low values of illumination range resistance for high values of illumination (large photocell currents) and vice versa, because their product is limited by the magnitude of the balancing voltage across the potentiometer. Thus uniform characteristics are obtained for wide ranges of illumination. Experimental conditions were such that a linear response of the photocell to light intensity could be reasonably expected.

The stability and reproducibility of the filter photometer were evidenced not only by consistent readings over a period of several months but by securing reproducible values of transmittance even when changing the light intensity from that of 32- and 50-candlepower, 6-volt lamps to that of a 110-volt, 100-watt lamp and when changing the current and spectral sensitivity from those of Type 2 to Type 1 photronic cells. On several occasions the instrument was dismantled and reassembled; after alignment, the transmittance figures previously secured were reproduced.

Photometer Operation

1. The light source is illuminated by the 6-volt battery.
2. The 1.5-volt battery is turned on.
3. Distilled water is used for a reference solvent. With distilled water in the light beam and switch *S* in the zero absorption position, the galvanometer is adjusted to zero with the "zero" dial. If necessary, the illumination range resistance is changed to make this possible.
4. With switch *S* in the 100 per cent absorption position and the shutter now placed in the light beam, the galvanometer is adjusted to zero with the "100 per cent" dial. If necessary, the concentration range resistance is changed to make this possible.
5. 3 is rechecked.
6. With the unknown solution brought into the light beam and switch *S* in center or absorption position, the galvanometer is adjusted to zero with the absorption dial. The absorption dial is calibrated from 0 to 100 in 0.5 divisions and reads directly in per cent absorption. 100 − the dial reading equals per cent transmittance.

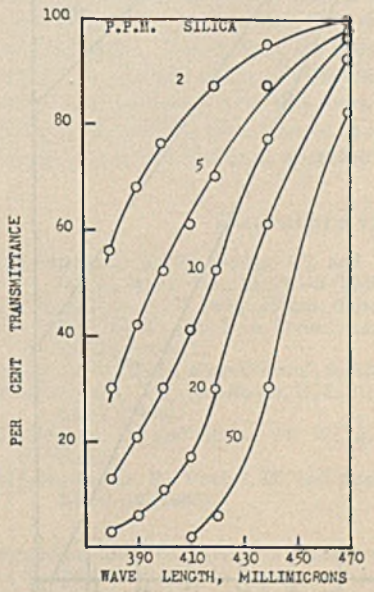

FIGURE 4. TRANSMITTANCE OF SILICOMOLYBDIC ACID

The two branches of the potentiometer circuit render it possible to make the full range of the 0–100 scale cover a narrower range of absorption than 0–100 if it is desirable to do so. The introduction of this feature necessarily complicates the circuit by the addition to the basic balancing circuit, in which the output voltage of the barrier layer cell across the illumination range resistor is balanced potentiometrically against a standardized drop, of another potential divider cir-

TABLE I. TRANSMITTANCE

Concentration	Transmittance[a] Full range 0–100%	Full range 0–24%
P. p. m. SiO_2	%	%
2	83.5	78
5	64.5	53
10	44	26
15	32	10

[a] Filter photometer; filter combination No. 511, 4 mm., and No. 038, 2 mm.; 50-mm. liquid depth; silica from sodium silicate.

TABLE II. TRANSMITTANCE OF SILICOMOLYBDIC ACID

Concentration	Wave Length, Millimicrons 390	400	410	420	440	470
	Transmittance[a]					
P. p. m. SiO_2	%	%	%	%	%	%
2	68	76	82	87	95	99.5
5	42	52	61	70	87	97.5
10	21	30	41	52	77	96
20	6	11	17	30	61	92
50	..	0.5	2	6	30	82

[a] Coleman double monochromator spectrophotometer, 19-mm. liquid depth, SiO_2 from sodium silicate.

cuit to establish a "false" zero for the barrier layer cell when the equivalent scale range is "spread". This is done by using a standard solution instead of the shutter in step 4—for example, 20 p. p. m. of silica show an absorption of 24 per cent. With such a solution in place (more conveniently done by using a buffered potassium chromate liquid standard for silica) the full range of the absorption dial covers 0–24 rather than 0–100. Characteristic results of such a test are shown in Table I.

As a reference instrument a Coleman double monochromater spectrophotometer was used.

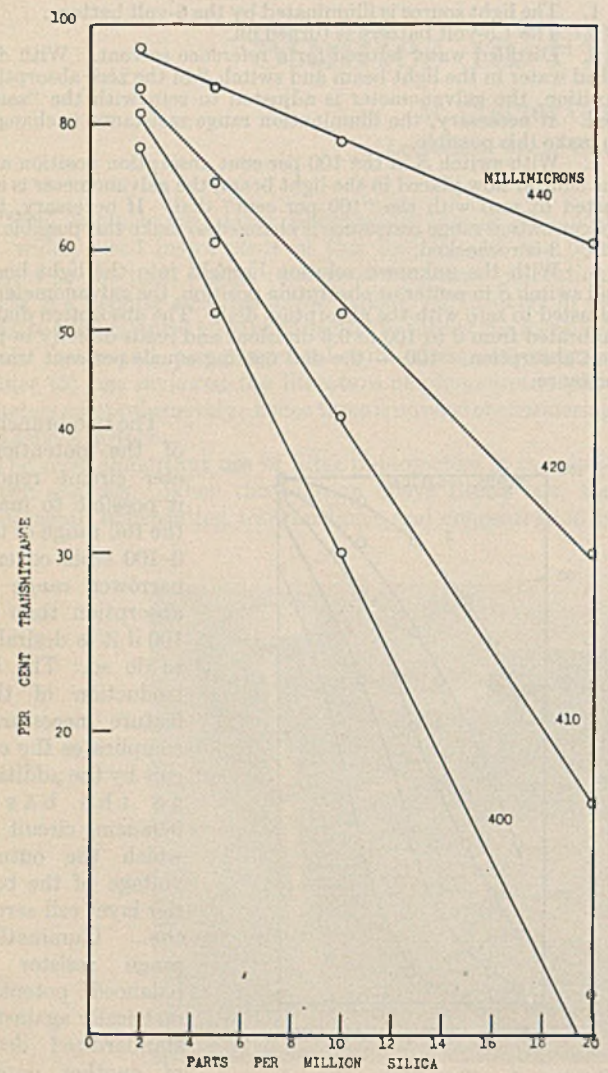

FIGURE 5

Reagents and Procedure

Ammonium molybdate solution, 10.0 grams of ammonium molybdate tetrahydrate per 100 ml. of distilled water.

Hydrochloric acid solution, one volume of concentrated acid to one volume of distilled water.

Sodium silicate solution, 3.0 grams of sodium carbonate fused with 0.2000 gram of pure, dry silica, dissolved in 200 ml. of distilled water, and diluted tenfold if desirable.

The ammonium molybdate and hydrochloric acid solutions are kept in Pyrex bottles; the sodium silicate solution is stored in a hard-rubber bottle.

To the 100-ml. sample are added and mixed 4 ml. of ammonium molybdate solution and 2 ml. of hydrochloric acid solution in rapid succession. It is advisable to wait 5 to 10 minutes for full color development.

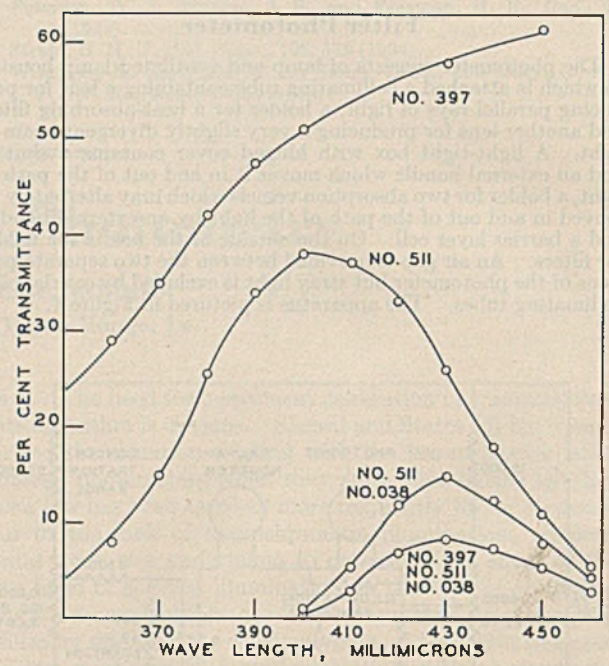

FIGURE 6. SPECTRAL TRANSMISSION OF FILTERS

As fundamental data the spectral transmittance of silicomolybdic acid, covering a reasonable range of concentration, was secured with the Coleman spectrophotometer (Table II and Figure 4). The data of Table II are replotted to give log per cent transmittance *vs.* concentration at constant wave length. As the data of Figure 5 show, the analytical linear calibration curves begin to deviate at wave lengths below 400 millimicrons. For maximum sensitivity it is preferable to work in the neighborhood of 410 millimicrons.

Several investigators using filter photometers have employed filters in this range of wave length. With the Pulfrich photometer for measuring instrument, Krumholz (*2*) used a 470 millimicron filter; Strohecker, Vaubel, and Breitwiesser (*7*) and Robinson and Spoor (*5*) used a 430 millimicron filter; while Pinsl (*4*) used a mercury vapor lamp and mercury (436) filter. Knudson, Juday, and Meloche (*1*) used the Corning No. 511, Cenco No. 1, and Evelyn No. 420 filters in a filter photometer. An examination of the Corning filter catalog indicated the use of filters 511 and 038. The data of Table

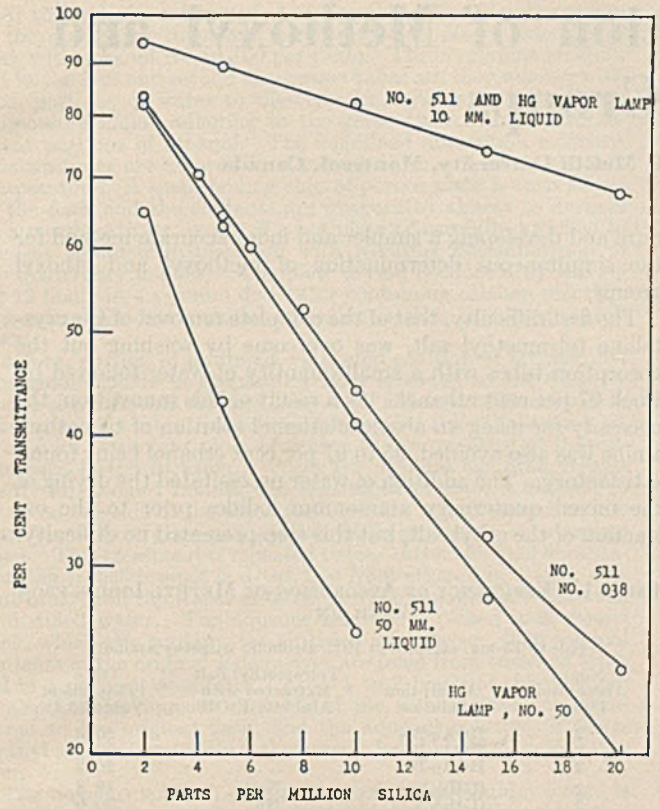

FIGURE 7

TABLE III. SPECTRAL TRANSMISSION OF FILTERS

Wave Length	Corning Filter Combinations: No. 511, 4 mm.	No. 511, 4 mm. + No. 038, 2 mm.	No. 397, 2 mm.	No. 511, 4 mm. + No. 038, 2 mm. + No. 397, 2 mm.
Millimicrons	Transmission[a] %	%	%	%
350	4.0	..	23.5	..
360	8.0	..	29.0	..
370	15.0	..	35.0	..
380	25.5	..	42.0	..
390	34.0	0.0	47.5	..
400	38.0	1.0	51.0	0.5
410	37.0	5.5	54.0	3.0
420	33.0	12.0	..	7.0
430	26.0	15.0	58.0	8.5
440	18.0	12.5	..	7.5
450	11.0	8.0	61.5	5.5
460	5.5	4.5	..	3.0
500	..	..	67.0	..

[a] Coleman double monochromator spectrophotometer.

III and Figure 6 present the spectral transmission of these filters, including the heat-absorbing filter No. 397, taken with the Coleman spectrophotometer.

The transmittance of silicomolybdic acid solutions, through a reasonable range of concentration, was determined with the filter photometer using various combinations of filters and light sources (Table IV). The data of Table IV are likewise replotted to give log per cent transmittance *vs.* concentration at different filter combinations. The data presented in Figure 7 show in accordance with information secured by the spectrophotometer that the filter combination Nos. 511 and 038, giving as they do a narrow band of light in the proper spectral region, give closest approach to a linear analytical curve when using filters. Somewhat better agreement using the mercury vapor lamp and filter is in further accord with the need for wave-length selection. This effect and the resulting

TABLE IV. TRANSMITTANCE OF SILICOMOLYBDIC ACID

Concentration	Filter Combinations: No. 511, 4-mm., 10 mm. of liquid	No. 511, 4-mm., 50 mm. of liquid	No. 511, 4-mm., No. 038, 2-mm., 50 mm. of liquid	Hg Vapor Lamp No. 50, 50 mm. of liquid	Hg Vapor Lamp No. 50, 10 mm. of liquid
P. p. m. SiO_2	Transmittance[a] %	%	%	%	%
2	94	65	83.5	83	95
4	..	..	70.5	..	..
5	89	43	64.5	63	89.5
10	82	26	44	41	82
15	74	..	32	28	..
20	..	..	24	20	68
25	..	..	19	..	..

[a] Filter photometer, SiO_2 from sodium silicate.

deviation above 10 p. p. m. become more noticeable as the solution thickness and concentration increase. The data furnished by the investigation show that the controlling factor in obtaining linear analytical calibration curves with a filter photometer is in securing the proper wave band of light; when the spectral region is properly defined for a particular analysis and the more nearly the spectral region isolated approaches the ideal, the more sensitive and in general the more nearly linear the calibration curves become. The effectiveness of a spectrophotometer at once becomes obvious and the apparent failure of a solution to obey Beer's law as obtained on many filter photometers is no doubt due, in part anyway, to improper wave-length selection, particularly in using wide wave bands.

The calculated specific extinction coefficient per 1 cm. of liquid thickness and per 1 p. p. m. of silica for the 511–038 filter combination is 0.007 (0.006 to 0.008) as compared to 0.006 for the comparable 440 millimicron data on the Coleman spectrophotometer. Strohecker, Vaubel, and Breitwiesser (7) obtained an average value of 0.010, using, however, different reagent strengths for developing the color reaction. The specific extinction coefficient for the 410 millimicron data on the Coleman spectrophotometer is 0.021 (0.020 to 0.023).

Acknowledgment

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Quantitative Determination of Methoxyl and Ethoxyl Groups

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THE determination of alkoxyl groups is based on their conversion into volatile alkyl iodides on treatment of an alkoxyl-containing compound with constant-boiling hydriodic acid. The direct determination of total alkoxyl, or of methoxyl or ethoxyl alone, is accomplished very readily by the Zeisel procedure (*3*) as modified by Vieböck (*4, 5*) and by Friedrich (*2*). In this procedure the alkyl iodide, after passing through a scrubber solution of cadmium sulfate and sodium thiosulfate (to remove hydriodic acid) is oxidized to iodate in a bromine–glacial acetic acid–potassium acetate solution (*5*) according to the following reactions:

$$Br_2 + RI \rightarrow RIBr_2 \rightarrow RBr + IBr$$
$$IBr + 2Br_2 + 2H_2O \rightarrow HIO_3 + 5HBr$$

The resulting iodate is then determined iodometrically by conversion to free iodine, which is titrated with sodium thiosulfate.

In order to differentiate between methyl iodide and ethyl iodide, the difference in solubility of tetramethyl ammonium iodide and of trimethylethyl ammonium iodide in alcohol is employed.

In the method of Willstätter and Utzinger (*6*), the alkyl iodides are absorbed in a 10 per cent absolute alcoholic trimethylamine solution. Although the reaction between trimethylamine and methyl iodide is rapid (98 per cent complete in 1 hour), that with ethyl iodide is very much slower; in consequence, the absorption solution is allowed to stand for at least 24 hours before the trimethylethyl ammonium iodide is separated from the tetramethyl ammonium iodide by extraction of the mixture of iodides with absolute alcohol. Each of the iodides is then determined gravimetrically by conversion to silver iodide with aqueous silver nitrate. When Wilson (*7*), however, showed that 100 cc. of absolute alcohol dissolve 0.040 gram of tetramethyl ammonium iodide at 25° C., Phillips and Goss (*3*) modified the Willstätter procedure by applying a correction for the solubility of the tetramethyl salt in the alcohol. In the Phillips and Goss procedure 100- to 300-mg. samples of known mixtures of *p*-ethoxybenzoic acid and anisic acid were used.

In order to avoid the necessity of correcting for the solubility of the tetramethyl salt in absolute ethanol, Wilson suggested the use of a saturated solution of tetramethyl ammonium iodide in this solvent as the trimethylethyl salt extractant. This modification has been incorporated in a tentative A. O. A. C. method for the determination of methanol in distilled liquors (*1*).

There are several disadvantages in Willstätter's (*6*) procedure for the determination of methoxyl and ethoxyl groups even after the above modifications are included. In the first place, during the hydrolytic stage of the process, the tetramethyl ammonium iodide separates in the absorption tubes in the form of minute crystals which tend to adhere firmly to the side of the tubes or flasks. Such crystals must be carefully separated with a rubber policeman or fine feather; the removal of those crystals adhering to the inside of the small-bore delivery tube is particularly difficult. Secondly, in order to isolate the ethyl derivative it is necessary to allow the absorption solution to stand for at least 24 hours. Even after this period, it is generally found that the quantity of trimethylethyl ammonium iodide isolated is somewhat less than the theoretical value. Finally, the yield of the two iodides is determined gravimetrically, either directly as the quaternary ammonium iodide (*1*) or as silver iodide.

The present investigation was carried out with the idea of eliminating the tedious, time-consuming steps in this procedure and developing a simpler and more accurate method for the simultaneous determination of methoxyl and ethoxyl groups.

The first difficulty, that of the complete removal of the crystalline tetramethyl salt, was overcome by washing out the absorption tubes with a small quantity of water followed by stock 97 per cent ethanol. As a result of this innovation, the necessity for using an absolute ethanol solution of trimethylamine was also avoided, 95 to 97 per cent ethanol being found satisfactory. The addition of water necessitated the drying of the mixed quaternary ammonium iodides prior to the extraction of the ethyl salt, but this step presented no difficulty.

TABLE I. EFFICIENCY OF ABSORPTION OF METHYL IODIDE FROM VANILLIN

(10- to 15-mg. samples in 10% alcoholic trimethylamine)

No. of Absorption Tubes	Absorption Solution	Tetramethyl Salt Extracted with Absolute EtOH	OMe (Theoretical Value 20.4)
2	HOAc-Br_2	....	20.3
2	HOAc-Br_2	....	20.2
2	HOAc-Br_2	....	20.2
2	$(CH_3)_3N$	No	19.8
2	$(CH_3)_3N$	No	20.0
2	$(CH_3)_3N$	Yes	19.9
2	$(CH_3)_3N$	Yes	19.7
2	$(CH_3)_3N$	Yes	19.3
2	$(CH_3)_3N$	Yes	19.9
2	$(CH_3)_3N$	Yes	19.9
3	$(CH_3)_3N$	No	20.3
3	$(CH_3)_3N$	No	20.0
3	$(CH_3)_3N$	No	20.2
3	$(CH_3)_3N$	Yes	20.3
3	$(CH_3)_3N$	Yes	19.7
3	$(CH_3)_3N$	Yes	20.0
3	$(CH_3)_3N$	Yes	20.2

The weakest point in the present methods for a combined methoxyl-ethoxyl determination lies in the fact that it is difficult to isolate the ethyl iodide quantitatively. Willstätter (*6*) showed that the formation of trimethylethyl ammonium iodide is 9 per cent complete in one hour, 77 per cent in 6 hours, and only 90 per cent in 24 hours. In order to avoid this difficulty it was found that the determination could be run in less time and with greater accuracy by first determining total alkoxyl according to the standard Vieböck-modified Zeisel procedure, then determining the methoxyl content alone according to the modified Willstätter procedure described below.

In order to simplify the actual determination of the tetramethyl ammonium iodide the gravimetric method was replaced by a volumetric method, using the same reagents and procedure employed in the total alkoxyl determination according to Vieböck (*4, 5*).

Procedure

METHOXYL DETERMINATION. A sample of 10 to 20 mg. (having from 10 to 40 per cent alkoxyl calculated as methoxyl) in a tin-foil microcontainer is added to the reaction vessel of the Zeisel apparatus containing the standard reaction mixture of constant-boiling hydriodic acid (5 cc.), phenol (2.5 grams), and a small piece of Nichrome wire. The procedure for this phase of the determination duplicates that of the standard Vieböck-Schwappach except for the substitution of a total of 15 cc. of a 10 per cent ethanol solution of trimethylamine as the absorption solution in place of the acetic acid–potassium acetate solution of bromine, and the use of three test tubes as receivers (the 15 cc. of absorption solution being divided into 6-cc., 5-cc., and 4-cc. portions, respectively).

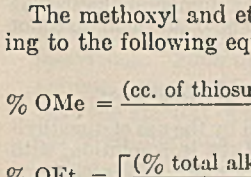
At the end of a reflux period of 50 minutes the liquid contents of the absorption tubes are transferred to a 50-cc. Erlenmeyer flask with ethanol (95 to 100 per cent). The crystalline residues left in the first and second absorption tubes are then washed with 2-cc. portions of water to dissolve the crystals of tetramethyl ammonium iodide adhering to the glass, then with two subsequent portions of ethanol. The combined absorption solutions plus washings are allowed to stand for at least 3 hours at room temperature. A small boiling chip of porous plate is then added to the flask and the contents are evaporated almost to dryness on a steam bath, or by use of a hot plate (if carefully watched to prevent complete evaporation with resulting decomposition of the quaternary iodides). Complete drying is effected by leaving for 12 hours in a vacuum desiccator containing calcium chloride. The trimethylethyl ammonium iodide is then extracted as follows:

A 3-cc. portion of a filtered, saturated solution of tetramethyl ammonium iodide in absolute ethanol is added to the flask by means of a medicine dropper pipet. (If absolute ethanol alone is used as extractant, a correction of 0.2 cc. of thiosulfate for 9 cc. of ethanol extractant must be added to the quantity of thiosulfate used in the titration.) After the mixture has been shaken gently for about 1 minute, the crystalline product is allowed to settle on one side of the tilted flask and the supernatant liquor is transferred to a glass-nail suction filter by means of a second pipet. This treatment is repeated twice. After the final decantation the transfer pipet is dried free from ethanol in a slow current of air, and the traces of tetramethyl compound are dissolved in distilled water. The aqueous solution is placed in a second flask, which also contains the air-dried filter paper. The residual contents of the original Erlenmeyer are freed from traces of ethanol in a vacuum desiccator. After 1 to 2 hours of this vacuum treatment, the aqueous contents of the second flask are transferred to the original flask, and the aqueous solution of tetramethyl ammonium iodide is then ready for volumetric determination.

The aqueous solution of the quaternary ammonium iodide is treated with 10 cc. of a saturated glacial acetic acid solution of potassium acetate containing 8 to 10 drops of bromine for 2 to 3 minutes, followed by the addition of 5 cc. of 25 per cent aqueous sodium acetate. The bromine is reduced with 10 drops of formic acid (80 to 90 per cent) and the resulting clear solution then treated with 5 cc. of 10 per cent aqueous potassium iodide solution and 5 cc. of 10 per cent sulfuric acid. The liberated iodine is titrated with standard (0.05 *N*) thiosulfate, using starch solution as an internal indicator.

Table II. Methoxyl-Ethoxyl Analyses

Compound	Found OMe	Found OEt	Calculated OMe	Calculated OEt
Vanillin	20.2	0.1	20.4	0.0
Vanillin	20.1	0.2	—	—
Vanillin	20.3	0.0	—	—
p-Ethoxybenzoic acid	0.0	27.0	0.0	27.2
p-Ethoxybenzoic acid	0.0	27.2	—	—
3-Ethoxy-4-methoxybenzoic acid	15.8	23.0	15.8	23.0
3-Ethoxy-4-methoxybenzoic acid	15.7	22.9	—	—
3-Ethoxy-4-methoxybenzoic acid	15.9	23.1	—	—
3-Ethoxy-4-methoxybenzoic acid	15.8	23.0	—	—
3-Ethoxy-4-methoxybenzoic acid	16.0	23.2	—	—
3-Ethoxy-4-methoxybenzoic ocid	15.9	23.1	—	—
3-Ethoxy-4-methoxybenzoic acid	16.0	23.2	—	—
3-Ethoxy-4-methoxybenzoic acid	15.7	22.9	—	—
α-Ethoxypropiovanillone (I)	13.7	20.2	13.8	20.1
	13.6	20.3	—	—
	13.7	20.2	—	—



The methoxyl and ethoxyl contents are calculated according to the following equations:

$$\% \text{ OMe} = \frac{(\text{cc. of thiosulfate}) \times (\text{normality of thiosulfate}) \times 31}{6 \times (\text{sample weight})}$$

$$\% \text{ OEt} = \left[\frac{(\% \text{ total alkoxyl as OMe}) - (\% \text{ OMe})}{31}\right] \times 45$$

Discussion of Results

Table I indicates that all the methyl iodide is collected when three receivers are used and that the yield of tetramethyl ammonium iodide is not affected by the ethanol extraction.

Table II includes the results obtained by this procedure with certain reference compounds.

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Recovery of Solvents Used in the Chemical Determination of Thiamine

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WHERE routine thiamine determinations are run by either the thiochrome method (*1*) or the colorimetric procedure (*2*) it is practical, and at the present time advisable, to recover the solvents, isobutanol in the former case and xylene in the latter. The distillation procedure generally used is tedious and requires attention, precautions must be observed to minimize fire hazards, and fluorescent materials in the isobutanol are not always completely removed.

The author has obtained isobutanol with blank readings much lower than those usually obtained by distillation in an all-glass still by treating waste solvent, containing thiochrome and other fluorescent materials, with activated vegetable charcoal (Darco-G-60, obtained from the Darco Corporation, 60 East 42nd St., New York, N. Y.). The isobutanol recovered in this manner has been used repeatedly in determining thiamine in a large variety of biological materials. Technical grade isobutanol, which usually contains fluorescent substances, may also be treated in this manner. The recovered isobutanol has proved to be as satisfactory in every respect as the solvent redistilled in all-glass apparatus; it fails to give rise to fluorescent substances on standing or after treatment with alkaline potassium ferricyanide or with alkali alone, according to the procedure for the estimation of thiamine.

The isobutanol, previously dried in the course of the assay, is recovered by shaking 100 cc. of solvent with 1.5 grams of activated charcoal for 15 to 30 minutes. This ratio of adsorbent to solvent is optimal, but equally good results are obtained by using an excess of charcoal—about 5 grams per 100 cc.—and shaking for only a few minutes. The charcoal suspension is then clarified by filtration. The filtrate, free from any fine particles of adsorbent, is used without further treatment.

In the Melnick-Field method for the determination of thiamine (*2*) the red color of the xylene solution is subjected to colorimetric evaluation. Here also, the solvent may be decolorized by the addition of activated charcoal, filtered, and used again.

The red dye complex in xylene appears to be adsorbed to the charcoal even more readily than the thiochrome from isobutanol solution. About 1 gram of charcoal is added to 100 cc. of the pigmented xylene solution and the suspension shaken for 5 minutes; this yields a colorless filtrate.

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Separation of Iron from Cobalt or Nickel

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THE ammoniacal ferricyanide method for the colorimetric determination of cobalt (*2*) requires the absence of practically all other metals. Using hydrogen sulfide, it is a simple matter to obtain the cobalt accompanied by only iron, nickel, manganese, chromium, aluminum, and zinc. The latter three may be removed by treatment with sodium peroxide, and the manganese by the use of the chlorate method (*4*). Nickel may be separated with dimethylglyoxime (*8*), but since the presence of iron requires careful control of the pH in this precipitation, a method was sought whereby either cobalt or nickel could be freed of iron, and a cobalt solution would be obtained ready for the colorimetric determination without the necessity of an intermediate reprecipitation of cobalt sulfide. With such a method of separation, the colorimetric method for cobalt could be easily applicable to steels, as well as to paint driers.

Experimental

In order to investigate published methods of separating mixtures of cobalt, iron, nickel, and manganese, solutions containing known amounts of these metals were prepared using Baker's C. P. chemicals. Methods of separating mixtures of all these metals were studied, but since it soon was evident that manganese and nickel could be separated quantitatively by accepted methods (*4, 8*), further work was concentrated on mixtures of cobalt and iron.

AMMONIUM HYDROXIDE PRECIPITATION. A solution containing 2 mg. of cobalt and 20 mg. of iron was subjected to successive reprecipitations with ammonium hydroxide. The filtrates were analyzed colorimetrically for cobalt (*2*), and the precipitates were tested by the Vogel test (*6*) for cobalt, yielding the following data:

	Cobalt	Vogel Test
	Mg.	
Original	2.0	.....
1st precipitation	0.7	Positive
2nd precipitation	0.45	Positive
3rd precipitation	0.00	Positive

Only 57.5 per cent of the original cobalt was recovered in the combined filtrates.

BASIC ACETATE SEPARATION. This method (*7*) was tried several times. Only once was the full amount of cobalt recovered, and the iron precipitate gave a negative Vogel test. Hence, though this method may be feasible under ideal conditions, it cannot be trusted for routine work.

ANTHRANILIC ACID SEPARATION. This method (*3*) was tried, again on the mixture of 2 mg. of cobalt and 20 mg. of iron. The precipitate was strongly contaminated with iron.

ALPHA-NITROSO-BETA-NAPHTHOL (*1*) SEPARATION. Since this reagent may precipitate iron as well as cobalt, it was not considered further.

HYDROCHLORIC ACID SEPARATION. In the qualitative analysis of Group III, cobalt and nickel sulfides are separated from the other sulfides and hydroxides by treatment with 1.2 *N* hydrochloric acid. However, no combination of time, temperature, or strength of hydrochloric acid could be found which dissolved all of the iron and none of the cobalt, for all ratios of these metals.

OXALIC ACID SEPARATION. As ammonium hydroxide is added slowly to a solution of cobalt sulfate, a precipitate of cobalt hydroxide forms, then dissolves in additional reagent. If the solution is boiled, enough ammonia can be expelled to permit a reprecipitation of the hydroxide. Thus, the contamination by cobalt of the ferric hydroxide obtained by ammonium hydroxide precipitation may be due to coprecipitation rather than occlusion.

If oxalates are present with the cobalt sulfate, slow addition of ammonium hydroxide does not form a precipitate at any time. Even after prolonged boiling precipitation still does not occur. Hence coprecipitation could be prevented by the formation of a cobalt oxalate complex.

Then 2.0 mg. of cobalt as the sulfate, with the amounts of iron shown below as the chloride, were diluted to 200 ml. and 10 ml. of a 10 per cent solution of oxalic acid were added. The iron was precipitated with ammonium hydroxide as usual and filtered. The filtrate was analyzed colorimetrically (*2*) and the precipitate tested for cobalt by the Vogel test.

Iron	Cobalt in Filtrate	Vogel Test
Mg.	*Mg.*	
2.0	2.00	Negative
10.0	2.00	Negative
20.0	2.00	Negative
50.0	1.45	Positive
2000	0.30	Positive

To determine the solubility of iron in the ammoniacal oxalate solution, 20 mg. of iron as the chloride were carried through the same procedure in the absence of cobalt. The filtrate was boiled down, the oxalate destroyed with sulfuric acid, and the residue analyzed for iron (*5*); 0.18 mg. were found. This quantity of iron does not interfere with the colorimetric determination of cobalt (*2*).

The use of 5 ml. of the oxalic acid solution gave poor separation in all cases. The use of 20 ml. of oxalic acid did not improve the separation. The maximum ratio of iron to cobalt which can be separated quantitatively by this method is shown to be 10 to 1. An alloy steel containing 15 per cent of cobalt could be analyzed directly by this means, but one containing 0.1 per cent could not be so treated.

In order to obtain the required ratio of iron and cobalt the ether-extraction method for iron chloride (*9*) was employed. A solution of 2.0 mg. of cobalt and 2000 mg. of iron in hydrochloric acid was so treated. This removed about 95 per cent of the iron, leaving 100 mg. A second ether extraction reduced the iron to about 10 mg. The iron-cobalt ratio then was 5 to 1, so the oxalic acid method was applied. The filtrate was found to contain 2.00 mg. of cobalt.

In the above work, the oxalic acid was found not to interfere with the colorimetric method used for the cobalt, except that crystallization occurred during the removal of volatile anions.

Description of Method

REAGENTS: A 10 per cent solution of oxalic acid and concentrated (15 *N*) ammonium hydroxide.

The sample should contain from 0.5 to 4 mg. of cobalt. If the ratio of iron to cobalt is greater than 10 to 1, successive ether extractions of the chloride (*9*) must be made until the ratio is reduced to 10 to 1.

To the acid solution containing cobalt and iron, in a volume of about 200 ml., 10 ml. of oxalic acid solution are added. The solution is neutralized with ammonium hydroxide, and 5 ml. excess are added. The precipitate is coagulated by boiling, then filtered and washed. All the cobalt will be in the filtrate.

If the cobalt is to be determined colorimetrically, the salt concentration must be controlled (*2*). To accomplish this, 6 ml. of 9 *N* sulfuric acid are added to the acid solution of cobalt and iron, and volatile anions are removed by evaporating to fumes of sulfur trioxide. The solution is cooled, diluted, and neutralized (litmus paper) with sodium hydroxide, then made just acid with sulfuric acid, and the volume is brought to about 200 ml. Then 10 ml. of oxalic acid are added, and the iron is precipitated with ammonium hydroxide, as described above. After filtration, the volume is reduced to 60 ml., during which the excess ammonia is expelled. The solution is then ready for the addition of the 10 ml. of potassium ferricyanide and 20 ml. of ammonium hydroxide required for the cobalt determination. The oxalic acid does not interfere.

Separation of Nickel and Manganese

A solution of nickel sulfate was analyzed by the use of dimethylglyoxime. Mixtures of this solution and the iron solution, with a ratio of iron to nickel of 10 to 1, were prepared. The nickel was precipitated from these mixtures with dimethylglyoxime, with careful pH control (*8*). The iron was precipitated by the above oxalic acid procedure, and the nickel in the filtrate precipitated with dimethylglyoxime, without careful pH control. The results of the two methods were identical. Ratios of iron to nickel greater than 10 to 1 were not investigated.

Mixtures of nickel, cobalt, and iron were prepared, and the iron was precipitated by the above procedure. The nickel in the

filtrate was precipitated as before, and quantitative recovery obtained. The cobalt then was determined colorimetrically. If this was attempted directly on the filtrate, the dimethylglyoxime was found to interfere with the color development. By precipitating the cobalt as sulfide, filtering, and then analyzing colorimetrically, however, quantitative recovery was proved.

Mixtures of nickel, manganese, iron, and cobalt were made and the iron was removed as described. The manganese divided between precipitate and filtrate. That in the precipitate did not interfere with the colorimetric determination of the iron. The nickel was removed from the filtrate with dimethylglyoxime, then the manganese with chlorate (*4*), and finally the cobalt was freed of all these reagents by precipitation as the sulfide, and determined colorimetrically. Again, quantitative recovery was obtained.

These experiments indicate that the separation of iron from cobalt or nickel by the use of oxalic acid is not interfered with by the presence of any other element normally present at that point in the analysis.

Summary

By the addition of oxalic acid before precipitation of ferric hydroxide by ammonium hydroxide, coprecipitation of cobalt and/or nickel is prevented, and a quantitative separation of these metals can be obtained.

The oxalic acid does not interfere with the subsequent colorimetric determination of either the cobalt or the iron, or the quantitative determination of nickel.

By this method, as little as 0.1 per cent of cobalt may be determined in a steel.

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Determination of Aliphatic Nitrate Esters

A Colorimetric Method

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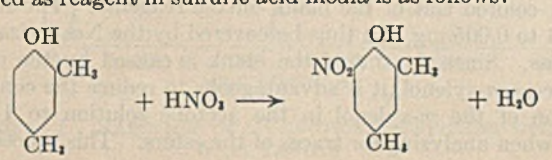
THE methods generally employed in the analysis of aliphatic nitrate esters are based on the estimation of the quantity of nitric or nitrous acid formed on the hydrolysis of the ester (*3*). While these methods are serviceable in the assay of small quantities of relatively pure ester material, it was not found practical to adapt the standard procedures to the analysis of biological samples containing nitroglycerine, erythritol tetranitrate, or pentaerythritol tetranitrate. Ethereal extracts of such samples are accompanied by other ether-soluble components such as fats and pigments which interfere with the colorimetry.

The method for the determination of inorganic nitrates described by Blom and Treschow (*1*), based on the formation of 5-nitro-4-hydroxy-1, 3-dimethylbenzene, seemed a practical approach to the solution of this problem, as the chromogenic compound is readily isolated from other substances interfering with the colorimetry by means of a steam-distillation. Experiments demonstrated that in the case of the three esters employed in the physiological studies, complete hydrolysis could be effected at room temperature by the action of 62.5 per cent sulfuric acid, and that the nitric acid formed could be determined quantitatively by the simultaneous nitration of *m*-xylenol. Subsequent experiments proved that the reactions proceeded in exact stoichiometric proportions and that the amount of ester present could be evaluated with the aid of an inorganic nitrate standard.

The reaction between nitric acid and the *m*-xylenol employed as reagent in sulfuric acid media is as follows:



The nitration product is readily volatile in steam and reacts with alkalies, forming an intense yellow-colored solution that obeys Beer's law. This reaction was utilized by Treschow and Gabrielsen (*5*) in the analysis of plant and soil extracts, and as a means of estimating inorganic nitrates in meat products by McVey (*2*). The sensitivity of the reaction was investigated by Werr (*6*), who found that in the absence of materials that interfered with the nitration process, 1 microgram of inorganic nitrate could be detected at a dilution of 1 part per million of sample solution.

The substances interfering with the reaction are large amounts of halogen salts, metals that liberate hydrogen with the sulfuric acid medium, nitrites, sulfides, and hydrogen peroxide. From the viewpoint of the proposed method for the analysis of nitrate esters, inorganic nitrates also constitute an interference. In the application of the method these interfering substances are of little importance, as the nitrate esters can be separated from them by an ether extraction. Traces of chlorides and inorganic nitrates which may dissolve in the ether layer are readily removed by washing the extract with 10 per cent sodium sulfate. Hydrogen peroxide will also dissolve in the ether layer, but its occurrence in appreciable quantity is not likely in samples of biological origin. When an extraction process is not employed, any sulfides or halogen salts present in the aqueous sample can be removed by precipitation with silver sulfate. Also, the traces of nitrite present in blood and urine would be destroyed in sulfuric acid media by the action of the urea and amino acids normally present in such samples (*4*).

Method of Analysis

REAGENTS. Sulfuric acid 62.5 per cent by volume, (sp. gr. 1.63) prepared by mixing 5 volumes of nitrate-free concentrated sulfuric acid with 3 volumes of water.

m-Xylenol, 2 per cent (Eastman preparation No. 1150, 4-hydroxy-1,3-dimethylbenzene) dissolved in acetone.

Sodium hydroxide solution, 2 per cent.

Potassium nitrate, 0.02 per cent.

The nitrate esters employed in the development of the method were of commercial origin. The erythritol tetranitrate and the pentaerythritol tetranitrate were purified by dissolving the compounds in warm acetone, filtering off insoluble matter, and diluting the filtrates with water at 50° C. The granular precipitates were filtered, washed with a mixture of acetone and water (1 + 3 by volume), and dried over calcium chloride in a vacuum desiccator. The nitroglycerine was not purified, but the commercial 10 per cent acetonic solution was assayed by evaporating off the solvent at room temperature and drying the residue to constant weight in a vacuum desiccator. Acetonic solutions of these preparations containing known amounts of the esters were employed in the development of the method.

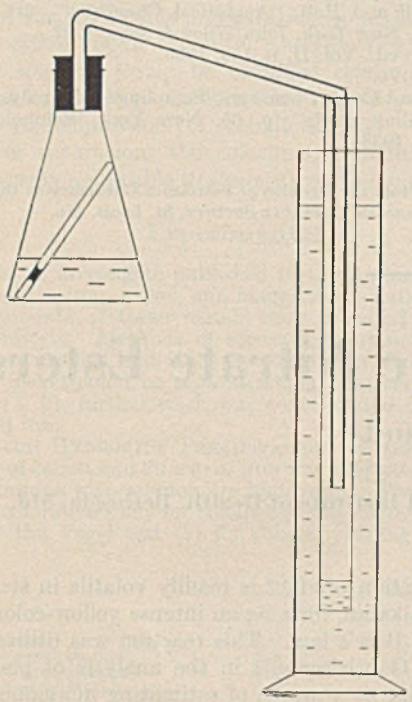


FIGURE 1. APPARATUS FOR DISTILLATION OF NITROXYLENOL

PREPARATION OF THE SAMPLE. The sample of blood, urine, or stomach contents is extracted with ether and the extract washed with 10 per cent sodium sulfate until the wash liquor gives a negative chloride test with silver nitrate. The ether layer is transferred to a volumetric flask and an aliquot portion containing not more than 3 mg. of the ester is withdrawn to a 300-ml. cone flask. The ether is evaporated off with the aid of a gentle stream of cold air. Warming of the solvent and prolonged aeration are to be avoided, as the esters, particularly nitroglycerine, are easily volatilized. The residue need not be perfectly dry and the aeration should be stopped when the bulk of the ether has volatilized. The residue is treated with 1 ml. of a 2 per cent acetonic solution of *m*-xylenol. The use of the acetone as a solvent for the *m*-xylenol is of fundamental importance, as it redissolves the ester and facilitates the quantitative progress of the nitration. This is of particular importance in the case of pentaerythritol tetranitrate which is extremely insoluble in water and gives low results in the absence of the acetone solvent. The solution is then treated with 15 ml. of 62.5 per cent sulfuric acid and allowed to react at room temperature for 30 minutes. At the termination of this period the mixture is diluted with 100 ml. of water and the nitroxylenol is separated by distillation.

DISTILLATION. If the distillation is conducted with an efficient water-cooled condenser, the nitroxylenol frequently solidifies on the condenser tube. This difficulty is overcome by conducting the steam directly into a water-cooled Nessler tube (50-ml., A.P.H.A. tall-form), as shown in Figure 1. The delivery tube is constructed so that the terminus is slightly above a 20-ml. calibration mark on the Nessler tube when the latter is buoyed up against the inclined arm. In the presence of fatty matter it is desirable to place a boiling rod and some porous tile in the flask to minimize bumping. The steam is collected in 5 ml. of 2 per cent sodium hydroxide, introduced into the tube so that the walls are wetted by the alkali. When the solution comes to a boil, the flame is reduced to a height of 2 to 3 cm. and 15 ml. of distillate are collected. The delivery tube is separated from the flask, and the lower arm is rinsed, allowing the wash water to run into the Nessler tube. The tube is cooled to room temperature, diluted to the 50-ml. mark, and the contents are mixed. The resultant yellow color is read in a Duboscq colorimeter, using 5-cm. cups against a standard solution of nitroxylenol prepared from a known weight of potassium nitrate.

Quantities of the esters over the range of 3.0 to 0.1 mg. can be matched against a standard prepared from 1 mg. of potassium nitrate. The standard is prepared by evaporating 5 ml. of 0.02 per cent potassium nitrate to near dryness, cooling, and reacting the residue with 1 ml. of the *m*-xylenol reagent and 15 ml. of 62.5 per cent sulfuric acid. This mixture is distilled after 30 minutes, following the same procedure as in the case of the samples. One milligram of potassium nitrate is equivalent to 0.7486 mg. of nitroglycerine, 0.7470 mg. of erythritol tetranitrate, and 0.7817 mg. of pentaerythritol tetranitrate.

TABLE I. DETERMINATION OF NITROGLYCERINE

Potassium Nitrate Reading[a]	Nitroglycerine		
	Taken	Recovered	Difference
Mg.	*Mg.*	*Mg.*	*Mg.*
3.81	3.00	2.85	−0.15
2.70	2.00	2.02	+0.02
1.26	1.00	0.94	−0.06
0.65	0.50	0.49	−0.01
0.26	0.20	0.19	−0.01
0.19	0.10	0.14	+0.04

[a] 1 Mg. of KNO_3 = 0.7486 mg. of $C_3H_5(NO_3)_3$.

TABLE II. DETERMINATION OF ERYTHRITOL TETRANITRATE

Potassium Nitrate Reading[a]	Erythritol Tetranitrate		
	Taken	Recovered	Difference
Mg.	*Mg.*	*Mg.*	*Mg.*
3.88	3.00	2.90	−0.10
2.70	2.00	2.02	+0.02
1.30	1.00	0.97	−0.03
0.70	0.50	0.52	+0.02
0.23	0.20	0.17	−0.03
0.14	0.10	0.10	0.00

[a] 1 mg. of KNO_3 = 0.7470 mg. of $(CHNO_3CH_2NO_3)_2$.

TABLE III. DETERMINATION OF PENTAERYTHRITOL TETRANITRATE

Potassium Nitrate Reading[a]	Pentaerythritol Tetranitrate		
	Taken	Recovered	Difference
Mg.	*Mg.*	*Mg.*	*Mg.*
3.90	3.00	3.04	+0.04
2.56	2.00	2.00	0.00
1.50	1.20	1.17	−0.03
1.30	1.00	1.02	+0.02
0.85	0.60	0.66	+0.06
0.24	0.20	0.19	−0.01

[a] 1 mg. KNO_3 = 0.7817 mg. of $C(CH_2NO_3)_4$.

The results obtained in the analysis of known quantities of pure nitrate esters are summarized in Tables I to III. The data reveal that the reaction proceeds in stoichiometric proportions within the limit of error of visual colorimetry. The average reproducibility of results in the analysis of pure ester is 1 per cent. In the application of the method to ether extracts of the digestive tracts of animals fed the esters in question, analyses of successive aliquots are reproducible within 3 per cent. The results of these physiological studies will be published in another article.

By viewing the color of the sodium salt of the nitroxylenol through the 23-cm. length of the 50-ml. Nessler tube it is possible to differentiate 5 micrograms of the esters from the straw-colored tint of the blank on the reagents. The range of 0.1 to 0.005 mg. can thus be covered by the Nesslerization process. Since the tint of the blank is caused by the color of excess *m*-xylenol, it is advantageous to reduce the concentration of the *m*-xylenol in the acetone solution to 1 per cent when analyzing for traces of the esters. This procedure

has been employed in measuring the solubility of the aliphatic nitrate esters in water and oil. It should prove useful in the analysis of air samples from the atmosphere of plants engaged in the manufacture of the esters.

Summary

A colorimetric method for the determination of aliphatic nitrate esters is described, based on the hydrolysis of the ester in 62.5 per cent sulfuric acid and the nitration of *m*-xylenol by the nitric acid liberated. The method has been applied to nitroglycerine, erythritol tetranitrate, and pentaerythritol tetranitrate over a range of 3.0 to 0.005 mg. The nitroxylenol is readily volatilized by a steam-distillation which permits the application of the method to complex systems. The reaction proceeds in stoichiometric proportions, so that the quantity of ester present can be evaluated from a potassium nitrate standard.

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Determining Free and Acetylated Sulfanilamide

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THE most commonly used procedure of analysis for sulfanilamide in solutions where both the free and acetylated forms are likely to be present is that of Bratton and Marshall (*2*). This method permits a fairly rapid determination of sulfanilamide, but in order to determine the total amount of sulfa drugs present, it is necessary to hydrolyze the acetyl component by heating in boiling water for an hour. This becomes rather tedious when only one sample is to be analyzed.

During a recent investigation in this laboratory it became necessary to determine the ultraviolet absorption spectrum of sulfanilamide. The results obtained indicated that the difference in the absorption spectra of the two forms of the drug could be utilized in performing a rapid analysis of solutions where both free and acetylated sulfanilamide were likely to be present. With this in mind, the extinction coefficients of the two compounds were determined and an apparatus was constructed for making the analysis of aqueous solutions. The results are presented in this paper, together with a summary of certain modifications that will be necessary if attempts are made to adapt the method for use in an instrument to determine the concentration of sulfanilamide in the blood.

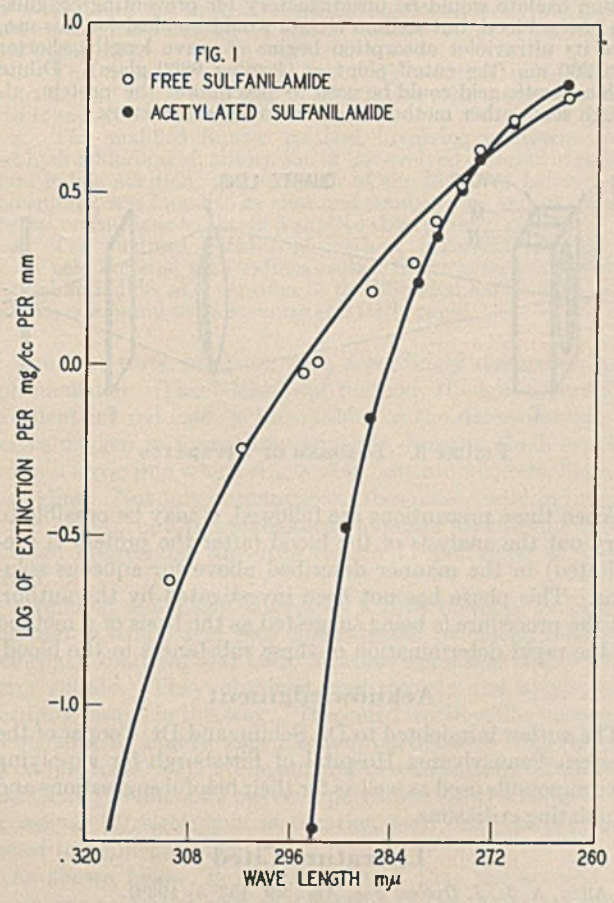

FIGURE 1. VARIATION OF EXTINCTION COEFFICIENT WITH WAVE LENGTH

Ultraviolet Absorption Spectra

The extinction coefficients of both free and acetylated sulfanilamide have been measured photographically in the spectral range 320 to 260 mμ.

For this work a Cenco replica grating spectrograph was employed, taking the spectra on Eastman Panchromatic process film. A hydrogen arc of the Allen (*1*) type furnished a source of an ultraviolet continuum. The absorption spectra were determined with solutions varying in concentration from 0.2 to 1.25 $\times 10^{-3}$ mg. per cc. These were placed in an absorption cell 13.7 mm. long equipped with Vycor windows. A step plate was added for calibration, and relative light intensities were determined with the aid of a Leeds & Northrup Speedomax recording microphotometer. Following Beer's law, $(\log I/I_0)/L$ was plotted against the concentration for a number of wave lengths in the region investigated. The negative slope of the resultant line gave the specific extinction in units of mg. per cc. per mm. when the concentration was expressed in mg. per cc. and the cell length, L, in millimeters.

A plot of the extinction coefficients as a function of wave length is shown in Figure 1 and the transmission spectra of the two compounds appear in Figure 2. Here spectrum 5 is for free sulfanilamide and spectrum 3 is for the acetylated form. From these we see that free sulfanilamide absorbs all light of wave lengths shorter than 310 mμ, while the acetyl derivative absorbs only wave lengths less than 286 mμ. Furthermore, the two compounds possess almost equal extinction coefficients for light in the range 282 to 260 mμ.

Since a difference exists between the spectra of the two forms of sulfanilamide, it was felt that this characteristic might be utilized in a rapid determination of the two compounds in solutions where both might be present together. This has been done for aqueous solutions. There should be no difficulty in extending the method to the corresponding analysis in blood solutions, provided certain necessary precautions are taken.

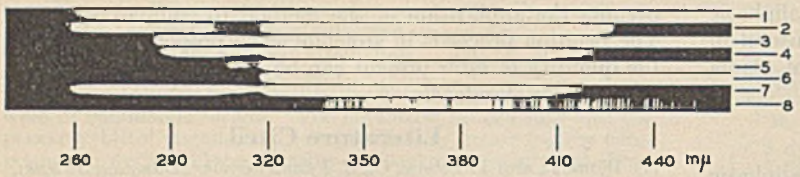

FIGURE 2. TRANSMISSION SPECTRA

Made with Cenco replica grating spectrograph (absorption occurring at 316 mμ in all spectra is due to transmission of silver coating on grating) using Eastman Panchromatic process film. Hydrogen arc used for ultraviolet continuum.

1. Transmission of water in Vycor Cell (limited in ultraviolet here by absorption of grating)
2. Transmission of Corning 9860 plus Corning 9840
3. Transmission of acetylated sulfanilamide
4. Transmission of Corning 9860 plus 0.125 inch of benzene
5. Transmission of sulfanilamide
6. Transmission of Corning 014
7. Transmission of Corning 9860
8. Brass arc

TABLE I. RECOVERY OF KNOWN AMOUNTS OF SULFA DRUGS IN WATER

Solution No.	Composition of Synthetic Solution: Sulfanilamide	Composition of Synthetic Solution: Acetylated sulfanilamide	Composition of Synthetic Solution: Total sulfa drug present	Concentrations Determined: Sulfanilamide	Concentrations Determined: Total sulfa drug	Recovery: Sulfanilamide	Recovery: Total sulfa drug
	Mg./cc.	*Mg./cc.*	*Mg./cc.*	*Mg./cc.*	*Mg./cc.*	%	%
1	0.05	0.0	0.05	0.053	0.053	106	106
2	0.025	0.025	0.05	0.028	0.046	111	92
3	0.025	0.0125	0.0375	0.024	0.041	96	109
4	0.05	0.0125	0.0625	0.047	0.069	94	110
5	0.0125	0.025	0.0375	0.013	0.032	104	86

Mode of Analysis

The apparatus used for the analysis is shown in Figure 3. It consists of a source, *A*, rich in lines between 310 and 260 mμ, and a glass indicator (014) which fluoresces when absorbing light of this wave length. The sample is placed in one side of the cell, *C*, and the concentration of sulfanilamide in the other side is varied until the two fluorescent fields show the same intensity. By interchanging the filters, *F*, we can differentiate the free sulfanilamide from the acetylated form.

The principles employed in executing the analysis can be understood most clearly by reference to Figure 2, which contains the transmission spectrum of each component of the instrument.

Spectra 5 and 3 are those of sulfanilamide and the acetyl derivative. Spectrum 6 is that of Corning 014 glass which fluoresces in the blue under the influence of the light it absorbs. The absorption range of the latter is almost identical with that of free sulfanilamide. It can be used, consequently, as a detector to determine the amount of light absorbed by the drug. A piece of Corning 9860 glass was placed before the arc in order to cut out the visible spectrum, and its absorption spectrum is shown as No. 7. When this is used with a 0.3-cm. (0.125-inch) cell of benzene we get spectrum 4. This combination, F_1, absorbs all the light below 280 mμ. Spectrum 2 is the transmission of Corning glass 9840. This filter in combination with Corning 9860 absorbs the light between 320 and 285 mμ and has the transmission of 9860 below 285 mμ. The set is employed as filter F_2.

In performing the analysis, a shield was used to shut off from view all but the lower part of the fluorescing field, *J*. The sample to be analyzed was placed in one side, *M*, of cell *C*, and 2 cc. of water were introduced into the other side, *N*. (The partition between the two sides of the cell was very thin, so that the two halves of the field, *J*, were nearly conterminous.) With filter F_1 at *F* the fluorescent fields received light of wave lengths that would be absorbed only by free sulfanilamide. Next, measured quantities of free sulfanilamide solution were added to *N* until fields *J* had the same visual intensity. The concentration of free sulfanilamide was then the same in both branches of the cell and could be calculated.

By substituting F_2 for F_1, only the light that was absorbed by both types of the drug was transmitted; hence if the sample contained any acetylated sulfanilamide, the fluorescing field in side *M* would be weaker than the one in *N*. It was assumed that both the free and acetylated forms had the same extinction in the spectral range transmitted, and more of the free sulfanilamide solution was added to *N* until balance was again achieved. Within the limits of this assumption the total sulfa drug concentration in side *M* was then equal to the free sulfanilamide concentration in *N* and was calculated. By subtraction the amounts of free and acetylated sulfanilamide were both known.

Aqueous solutions containing free and acetylated sulfanilamide were analyzed in this manner. An instrument was improvised from available equipment, employing the crude optical design of Figure 2 and a cell capacity of 3.5 ml. in each side. With mixtures of free sulfanilamide and acetylated sulfanilamide in which the total sulfa drug concentration was of the order of 0.05 mg. per cc., an accuracy of ±10 per cent was obtained in determining the amount of free sulfanilamide (filter F_1 at *F*), and of ±15 per cent in estimating the total sulfa drug content (filter F_2 at *F*). One set of data is shown in Table I. The accuracy could be increased appreciably, however, by improving the optical design and adjusting the cell size. The accuracy was limited largely by the exactitude with which the solution volumes could be measured. The total time required for analysis varied between 3 and 5 minutes.

In order to extend the method to the determination of these drugs in the blood, it will be necessary to observe certain precautions.

When drawing the blood and precipitating the protein, it is essential to prevent the introduction of compounds whose absorption spectra overlap the region 320 to 260 mμ. To illustrate, sodium oxalate would be unsatisfactory for preventing coagulation of the blood, but sodium citrate would be ideal for this use, since its ultraviolet absorption begins at wave lengths shorter than 260 mμ (the cutoff point of Corning 9860 glass). Dilute trichloroacetic acid could be used to precipitate the protein, although some other method might be more satisfactory.

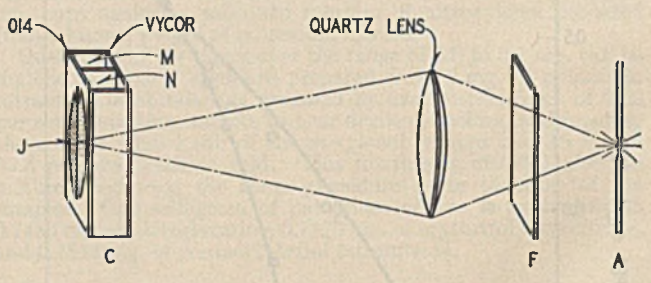

FIGURE 3. DIAGRAM OF APPARATUS

When these precautions are followed, it may be possible to carry out the analysis of the blood (after the protein is precipitated) in the manner described above for aqueous solutions. This phase has not been investigated by the author, but the procedure is being suggested as the basis of a method for the rapid determination of these substances in the blood.

Acknowledgment

The author is indebted to Dr. Schinn and Dr. Cooper of the Western Pennsylvania Hospital of Pittsburgh for supplying the compounds used as well as for their helpful suggestions and stimulating criticisms.

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Determination of Active Oxygen in the Presence of Barium and Lead

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DURING the past two years, the Chemical Laboratory of the Geological Survey has made a number of complete analyses of manganese minerals, in connection with studies of ores of manganese, a strategic metal. One of the determinations required is the percentage of "active" or "available" oxygen. This is usually easily made by adding to a weighed sample an excess of standard oxalic acid or sodium oxalate solution plus a little sulfuric acid, heating until solution of the mineral is complete, and back-titrating the excess oxalate with potassium permanganate.

This method, however, is inapplicable to the analysis of the barium manganate minerals psilomelane and hollandite and the lead manganate mineral coronadite. Psilomelane is of very common occurrence, and the other two minerals are occasionally encountered. When these minerals are heated with oxalic and sulfuric acids, the precipitated barium or lead sulfate coats undissolved particles of the mineral and prevents them from reacting, with consequent low results for active oxygen. For the same reason, this method cannot be used for the determination of active oxygen in lead dioxide (PbO_2), in red lead (Pb_3O_4), or in barium or strontium peroxides.

Several methods have been proposed for this determination. Four of these were studied in 1937 by Mrgudich and Clark (*1*), whose results may be summarized briefly:

1. The Lux method, involving solution in oxalic acid and nitric acid and the back-titration with permanganate, was found to be inaccurate because of a side reaction between nitric acid and oxalic acid.
2. The Schaeffer method, involving solution in hydrogen peroxide and nitric acid and back-titration with permanganate, was found to be inaccurate.
3. The modified Bunsen method, involving treatment with hot hydrochloric acid, absorption of the evolved chlorine in potassium iodide solution, and titration of the liberated iodine with thiosulfate, was found to be slow and cumbersome and to require special precautions to obtain complete absorption of chlorine.
4. The modified Diehl-Topf method, involving solution in acetic acid buffered with sodium acetate in the presence of excess potassium iodide, and titration of the liberated iodine with thiosulfate, was found to be accurate and fairly rapid.

The first three methods were accordingly discarded from consideration. The Diehl-Topf method, though apparently excellent for red lead, is unsuitable for the determination of active oxygen in manganese minerals, because these usually contain ferric iron which reacts with potassium iodide, liberating iodine. Not only do many manganese ores contain admixed ferric oxides, but the manganese minerals themselves often contain appreciable amounts (up to 11 per cent) of ferric oxide.

Mrgudich and Clark suggested a fifth method, involving solution in oxalic acid plus concentrated perchloric acid (50 per cent), dilution, and back-titration electrometrically with ceric sulfate. They obtained reproducible and apparently accurate results in this way. It seemed worth while, however, to attempt to modify their method, particularly with respect to two features: (1) The amount of acid used by Mrgudich and Clark (25 ml. of 72 per cent perchloric acid) seemed to be excessive; (2) electrometric titration with ceric sulfate happened to be inconvenient.

As shown below, it has been found that the amount of perchloric acid used may be diminished to less than one third the amount recommended by Mrgudich and Clark, and that the titration may be made with permanganate. There is no loss in accuracy or in speed, and no special technique is required.

TABLE I. BLANK RUNS

No.	0.06035 *N* Oxalic Acid *Ml.*	H_2SO_4 Concn. *Vol. %*	Treatment	Time *Min.*	0.0797 *N* $KMnO_4$ *Ml.*
15	50	5	80°	0[a]	37.84
18	50	5	Boiled	45	37.85
13	50	20	Boiled	45	37.82
25	50	30	80°	0[a]	37.88
22	50	30	Boiled	45	36.92
4	50	30	Boiled	45	36.76
		$HClO_4$ Concn.			
11	50	5	Boiled	45	37.83
9	50	20	Boiled	45	37.86
12	50	25	Boiled	45	37.80
21	50	30	80°	0[a]	37.87
14	50	30	Boiled	45	37.82
17	50	30	Boiled	45	37.48
26	50	50	Steam bath	60	37.83
27	50	50	Steam bath	45	37.84

[a] Heated to 80° and immediately titrated.

Experimental

A series of blanks was run with 50 ml. of 0.06035 *N* oxalic acid and varying amounts of sulfuric and perchloric acid in order to determine whether any side reaction occurred which consumed oxalic acid. Typical results, given in Table I, show that in sulfuric acid solution of moderate concentration there is no side reaction, but that some reaction which consumes oxalic acid occurs when 30 per cent sulfuric acid is boiled with oxalic acid for 45 minutes. This suggests the precaution of not allowing solutions to concentrate too far while the sample is being dissolved.

In perchloric acid solution, no appreciable side reaction occurred in acid of moderate concentration. When 30 per cent solutions of perchloric acid were boiled with oxalic acid, the titer was usually unaffected, but sometimes, as indicated, it was appreciably lower than the normal titer, as though some side reaction had occurred. It will be noted that 50 per cent solutions, heated at about 95° in the steam bath, showed normal titer. This is in agreement with the results of Mrgudich and Clark. The small amount of side reaction which perhaps occurred in 30 per cent acid may be due to the higher temperature during the heating, about 110° compared to 95° for the 50 per cent solution.

Table II shows some analyses by different procedures and the time required for complete solution. It is evident that neither speed nor accuracy is lost by the modifications in procedure suggested here; in fact, the 50 per cent acid seemed to require a little longer for complete solution of the mineral, perhaps because the solutions heated in the steam bath were relatively quiescent and less well mixed than the boiling solutions of lower acid concentration.

Mrgudich and Clark made comparative analyses of the same lead dioxide sample by different methods, but, as the purity of lead dioxide cannot be determined, they presented no data to show that the values obtained are the true figures.

TABLE II. ANALYSES

Material	Gram	Acid Used	%	Time for Solution Min.	Active Oxygen %
PbO_2	0.3300	$HClO_4$	5	55	6.18
PbO_2	0.3300	$HClO_4$	5	50	6.16
PbO_2	0.3300	$HClO_4$	30	48	6.17
PbO_2	0.3300	$HClO_4$	50	65	6.05
Pb_2O_4	0.7000	$HClO_4$	5	12	2.06
Pb_3O_4	0.7000	$HClO_4$	30	10	2.05
Pb_3O_4	0.7000	$HClO_4$	50	12	2.07
Cryptomelane (2)	0.1100	$HClO_4$	5	30	15.31
Cryptomelane (2)	0.1100	$HClO_4$	5	Overnight	15.26
Cryptomelane (2)	0.1100	H_2SO_4	5	25	15.29
Cryptomelane (2)	0.1100	$HClO_4$	50	40	15.33

TABLE III. DUPLICATE ANALYSES

	Active Oxygen 5% H_2SO_4 %	5% $HClO_4$ %
Cryptomelane (2)		
Urucum, Brazil	14.52	14.56
Philipsburg, Mont.	16.07	15.99
Deming, N. Mex.	15.92	15.94
Sugar Stick, Ark.	14.97	15.12
Pyrolusite, Lake Valley, N. Mex.	17.89	17.86
Pyrolusite, Cuba	16.32	16.30
Rancieite, Cuba	13.80	13.82

The last of the three samples in Table II contained no lead and only 0.13 per cent barium oxide, so that the determination could be made using sulfuric acid. The results with sulfuric acid and perchloric acid are in excellent agreement. A number of similar samples have been run in duplicate, using sulfuric acid for one and perchloric acid for the other, with the results shown in Table III. It is evident that the perchloric acid method gives correct answers for these samples and it is to be presumed that the values obtained on material containing lead or barium are also correct.

It seemed probable that phosphoric acid could also be substituted for sulfuric acid in this determination. Experiments showed, however, that the C. P. phosphoric acid available gave fading and rather uncertain end points, presumably caused by the presence of some impurity. A few determinations made with 5 per cent phosphoric acid gave results in approximate agreement with those obtained using perchloric acid. No attempt was made to purify the phosphoric acid.

Procedure Recommended

To an appropriate weight of sample (0.15 gram of barium or lead manganate, 0.3 gram of lead dioxide or 0.7 gram of red lead), which is preferably ground to 200-mesh, add from a pipet, which need not be calibrated, 50 ml. of approximately 0.05 *N* oxalic acid solution, 8 ml. of 60 per cent perchloric acid, and 42 ml. of water. Place a short-stemmed funnel in the mouth of the flask to act as a condenser, and boil gently until solution is complete (usually about 20 minutes, but as long as 90 minutes may be required for resistant minerals such as hollandite). Add water from time to time if necessary to prevent the volume from diminishing to less than 50 ml. Dilute to 100 ml. and titrate directly at 80° with approximately 0.07 *N* potassium permanganate. The blank experiment to determine the permanganate equivalent of the oxalic acid needs only to be heated to 80° and need not be boiled as long as the actual determination, since there is no loss of titer.

Standard sodium oxalate solution or a weighed amount of sodium oxalate may be substituted for the oxalic acid solution, but is less convenient to use. Some samples may contain dark insoluble material. In such cases, the completeness of solution of the mineral containing active oxygen may be judged by noting whether bubbles of oxygen come off the undissolved particles. It is convenient at times to place the flask covered with a watch glass on the steam bath overnight. Solution is complete and the results obtained are accurate.

Summary

The method of Mrgudich and Clark is modified by substituting 5 per cent (by volume) perchloric acid for 50 per cent perchloric acid. Titration by potassium permanganate may be substituted for electrometric titration with ceric sulfate.

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New Reagents for Sodium

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SODIUM reagents consisting of uranyl acetate and certain divalent metal acetates in dilute acetic acid solution have come into extensive use for the detection and determination of sodium. Unfortunately, all such reagents now in use are more or less sensitive toward lithium, so that they are not satisfactory for the detection or determination of sodium in the presence of appreciable quantities of lithium. An indication was found, however, in an investigation by Caley and Baker (*1*) that a sodium reagent containing uranyl acetate and cupric acetate is less sensitive toward lithium than other reagents of this general type. The value of such a reagent for the detection and determination of sodium in the presence of lithium was the principal subject of the present investigation.

Aqueous Cupric Acetate–Uranyl Acetate Reagent

By means of tests with a series of trial reagents in which the concentrations of the components were systematically varied, it was found that a reagent of the following composition gave the most satisfactory results:

Uranyl acetate dihydrate	88 grams
Cupric acetate monohydrate	88 grams
Glacial acetic acid	60 ml.
Water	To 1000 ml.

The salts are dissolved in the acetic acid and nearly all the necessary water at a temperature of 50° to 60° C., after which the solution is cooled to room temperature, adjusted to final volume with water, and allowed to stand a day. The solution is then maintained at 20° C. while being stirred vigorously for about 2 hours with a mechanical stirrer, and is next filtered through a dry filter to remove the small amounts of precipitated salts. The reagent prepared in this way is stable.

The sensitivity of this reagent toward sodium and lithium is indicated in Table I. Though it is evidently insensitive toward lithium, it is unfortunately also not very sensitive toward sodium. Experiments with the trial reagents showed that the sensitivity toward lithium can be further reduced by decreasing the concentration of uranyl acetate in the reagent, but unfortunately this is paralleled by a decrease in sensitivity toward sodium. An aqueous reagent of this type is obviously not satisfactory for the accurate quantitative determination of sodium, however useful it may be for the qualitative detection of sodium in the presence of considerable lithium.

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Table I. Sensitivity of Aqueous Cupric Acetate–Uranyl Acetate Reagent toward Sodium and Lithium

Ion	Amount Present	Reaction on Addition of Stated Volume of Reagent to 1 Ml. of Chloride Solution			
	Mg.	*1 ml.*	*3 ml.*	*5 ml.*	*10 ml.*
Na	2	+	+	+	+
	1	−	+	+	+
	0.5	−	+	+	+
	0.3	−	−	−	−
Li	20	+	+	+	+
	10	−	−	−	−

Alcoholic Cupric Acetate–Uranyl Acetate Reagent

By replacing part of the water in the aqueous reagent with ethyl alcohol and altering the proportion of the other components, it was found through systematic experiments that a reagent could be prepared that was at the same time more insensitive toward lithium and more sensitive toward sodium than the aqueous reagent. The composition of this alcoholic reagent is as follows:

Uranyl acetate dihydrate	40 grams
Cupric acetate monohydrate	25 grams
Glacial acetic acid	100 ml.
Ethyl alcohol, 95%	500 ml.
Water	450 ml.

The salts are dissolved in the water and acetic acid at a temperature of 50° to 60° C., and then after cooling down to room temperature the alcohol is added with constant stirring. After standing at least one day, preferably 2 or 3, the mixture is stirred and filtered in the same way as described for the preparation of the aqueous reagent. The longer time of standing in the preparation of the alcoholic reagent is recommended because the excess of salts separates more slowly than in the preparation of the aqueous reagent. This alcoholic reagent is somewhat less stable than the aqueous, particularly in the presence of light. However, a specimen of this reagent which had been protected from strong light was still effective a year after its preparation.

In Table II is indicated the sensitivity of this reagent toward sodium and lithium. As little as 0.1 mg. of sodium may be detected if sufficient volume of reagent is used, and as much as 50 mg. of lithium will not produce a precipitate when the volume of reagent amounts to 20 ml. With this same volume of reagent as much as 40 mg. of potassium present as chloride will also not produce a precipitate. When, however, potassium is present as sulfate less potassium will result in the formation of a precipitate, in part because of the low solubility of potassium sulfate in strong alcoholic solution. This and similar interference from the low solubility of a salt in alcoholic solution may be obviated by adding alcohol to the test solution until the concentration is about 50 per cent, filtering off any precipitate, and then adding the reagent. When the test is performed in this fashion 0.1 mg. of sodium present in the original test solution may still be detected. Ammonium alone even in high concentration does not produce a precipitate with this reagent. The effect on the sensitivity of the reagent toward sodium when other alkali ions are also present is indicated in the tables and discussion on the quantitative application of this reagent.

Anions such as ferrocyanide or phosphate which precipitate copper or uranium interfere with the detection of sodium with this reagent. The presence of much free strong acid appreciably reduces the sensitivity of this reagent toward sodium—for example, even 1 mg. of sodium produces no precipitate when the test solution is 6 *N* in hydrochloric acid.

This reagent is stable for as long as a year if kept out of strong light. It rapidly deteriorates if exposed to sunlight because of photochemical reduction of the uranyl salt. Because of this the reagent should always be kept in a dark bottle.

Composition of Sodium and Lithium Precipitates

By analogy with the triple acetates produced by other sodium reagents, it is to be expected that those produced by this aqueous cupric acetate–uranyl acetate reagent would have the composition

$$3UO_2(C_2H_3O_2)_2.Cu(C_2H_3O_2)_2.MC_2H_3O_2.XH_2O$$

where M is sodium or lithium and X approximately 6.

Samples of the sodium salt were prepared by precipitating 20 mg. of sodium contained in 2 ml. of solution with 20 ml. of the aqueous reagent, filtering the precipitated salt on a glass crucible, washing it sparingly with 95 per cent ethyl alcohol, and drying in air to constant weight. In order to prepare sufficient lithium salt for analysis, it was necessary to have 250 mg. of lithium present in 5 ml. of solution, and to add 25 ml. of reagent. The lithium salt was washed and dried in the same way.

The composition of the sodium triple salt was checked by copper and uranium determinations. Samples were dissolved in dilute sulfuric acid, and, after being evaporated to fumes of sulfur trioxide and diluted, the solutions of the samples were electrolyzed for the determination of copper. The uranium in the filtrates from the electrolyses was precipitated as ammonium diuranate by means of carbonate-free ammonium hydroxide and weighed as U_3O_8. The analyses of the lithium triple salt included determinations of the lithium content, lithium being determined in the filtrates from the uranium separations by evaporating with dilute sulfuric acid in a platinum dish and weighing the residue as Li_2SO_4. Because of the method of analysis it is likely that these lithium determinations were a little high.

Table II. Sensitivity of Alcoholic Cupric Acetate–Uranyl Acetate Reagent toward Sodium and Lithium

Ion	Amount Present	Reaction on Addition of Stated Volume of Reagent to 1 Ml. of Chloride Solution				
	Mg.	*1 ml.*	*3 ml.*	*5 ml.*	*10 ml.*	*20 ml.*
Na	0.3	+	+	+	+	+
	0.2	−	+	+	+	+
	0.1	−	−	+	+	+
Li	50	+	+	+	+	−
	25	−	−	−	−	−

Table III. Analyses of Precipitates

(Produced by adding an aqueous cupric acetate–uranyl acetate reagent to solutions containing sodium or lithium)

	Copper %	Uranium %
Sodium Precipitate		
Found	4.15	46.50
	4.16	46.50
Calculated for a hexahydrate	4.14	46.50
	Lithium %	
Lithium Precipitate		
Found	0.55	4.21 46.90
	0.54	4.22 46.93
Calculated for a hexahydrate	0.45	4.16 46.79

The results of the analyses are shown in Table III. It is evident that both the sodium and the lithium salts precipitated by the aqueous cupric acetate–uranyl acetate reagent are of the same type as those precipitated by the other aqueous sodium reagents.

Although the precipitate produced by adding the alcoholic reagent to sodium solutions was found to be a triple salt of the same type as regards the ratios of component salts, this triple salt was, surprisingly enough, solvated with both water and alcohol. That alcohol is actually a chemically combined component of the salt was shown by the fact that it is retained in constant proportion in preparations dried at different elevated temperatures, and is retained even at temperatures above the boiling point of the alcohol. No sensible loss in weight

TABLE IV. ANALYSIS OF PRECIPITATE

(Produced by adding an alcoholic cupric acetate–uranyl acetate reagent to solutions containing sodium)

	Copper	Uranium	Alcohol
	%	%	%
Found	4.00	45.15	4.04
	4.02	45.22	4.07
	4.04	45.14	...
Calculated for Possible Solvated Salts			
I	4.02	45.13	2.91
II	3.98	44.74	4.33
III	4.00	44.99	4.35
IV	4.03	45.25	4.38
V	3.99	44.85	5.78

Key to Possible Solvated Salts

I. Triple salt solvated with 6 H_2O and 1 C_2H_5OH, M. W. = 1582.4
II. Triple salt solvated with 5½ H_2O and 1½ C_2H_5OH, M. W. = 1596.4
III. Triple salt solvated with 5 H_2O and 1½ C_2H_5OH, M. W. = 1587.4
IV. Triple salt solvated with 4½ H_2O and 1½ C_2H_5OH, M. W. = 1578.4
V. Triple salt solvated with 4 H_2O and 2 C_2H_5OH, M. W. = 1592.4

occurred on heating to 60° C. a preparation which had been dried to constant weight in air at room temperature. On heating this up to 85° C. a slight loss in weight occurred which amounted to 0.5 per cent when constancy was reached. This was only a small fraction of the alcohol content of the salt, and it is not certain whether this loss in weight was due entirely to escape of alcohol or to escape of both water and alcohol. On heating this same preparation to 110° C. a gradual loss in weight occurred extending over a period of 10 weeks. This loss in weight was accompanied by a general decomposition of the salt, as was shown by a color change from a tan to a deep green.

Samples of the sodium salt for analysis were prepared in a manner analogous to that employed in preparing the samples of sodium salt precipitated by the aqueous reagent, and the copper and uranium determinations were made in the same way. The determinations of the alcohol content were made on separate samples by oxidizing them for 1 hour at 100° C. with a measured amount of standard potassium bichromate acidulated with sulfuric acid, the excess of bichromate being determined on cooling by potentiometric titration with a freshly standardized solution of ferrous sulfate. It was not found possible to estimate the alcohol content by measuring the loss in weight on heating, since at a temperature at which the alcohol was driven off some water was also driven off. Nor was it found possible to estimate by the same means the total content of alcohol and water, since at the temperature required to drive both these off decomposition of the component acetates began. However, the total degree of solvation could be estimated from a rough determination of the molecular weight of the salt, this being done by precipitating quantitatively known weights of sodium and weighing the resulting precipitates. It was found that the molecular weight must lie between 1575 and 1600.

Results of the analyses and a tabulation of the composition of the various possible solvates are given in Table IV. This triple salt is solvated with about 1.5 molecules of alcohol and with 4.5 or 5 molecules of water. Subsequent to the work reported in this paper, determinations of the water content of this triple salt by means of the Karl Fischer reagent have indicated that the water content is close to 5 molecules (*4*). Thus the composition of the salt is reasonably well established.

If the assumption had been made that the salt was solvated with water only, and the degree of solvation had been estimated from the copper and uranium determinations, it would have been concluded that this salt was an octahydrate. This is interesting, inasmuch as Kahane (*3*) reported that sodium magnesium uranyl acetate precipitated by an alcoholic magnesium acetate–uranyl acetate reagent is an octahydrate. An analysis of samples of sodium magnesium uranyl acetate precipitated by Kahane's reagent showed that this salt is not an octahydrate as reported by Kahane on the basis of determinations of the metal and acetate content but is a salt solvated with both water and alcohol. Two determinations of the alcohol content gave 4.17 and 4.18 per cent. The sodium salt precipitated by an alcoholic magnesium acetate–uranyl acetate reagent is thus analogous in composition to that precipitated by the alcoholic cupric acetate–uranyl acetate reagent. Confirmation of the presence of alcohol as an essential component in the precipitate produced by Kahane's reagent was found soon after this work was completed in an abstract of a paper by Schoorl (*5*). The presence of alcohol in this precipitate is a source of error that must be taken into account if sodium is to be determined by reduction of the uranium in a solution of the precipitate followed by titration with an oxidizing agent, as has been recommended by Kahane and others, since results are likely to be high because of the presence of alcohol as a second oxidizable substance.

TABLE V. DETERMINATIONS OF SODIUM WITH ALCOHOLIC CUPRIC ACETATE–URANYL ACETATE REAGENT

Reagent	Sodium Chloride Solution	Sodium Present	Sodium Found	Difference
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*
50	2	1.0	0.9	−0.1
100	5	1.0	1.0	±0.0
25	5	10.0	9.2	−0.8
50	5	10.0	10.0	±0.0
100	5	10.0	10.1	+0.1
125	5	10.0	9.8	−0.2
50	2	20.0	19.7	−0.3
50	2	20.0	20.0	±0.0
100	2	20.0	20.0	±0.0
100	5	20.0	20.0	±0.0
100	2	50.0	47.8	−2.2
150	5	50.0	48.4	−1.6
250	5	50.0	49.7	−0.3
350	2	50.0	49.9	−0.1

Quantitative Application of Alcoholic Reagent

The alcoholic cupric acetate–uranyl acetate reagent may be used for the quantitative determination of sodium with satisfactory results. In the trial determinations reported here the procedure used was essentially that of Caley and Foulk (*2*) employing the aqueous magnesium acetate–uranyl acetate reagent, except that the precipitate was collected on glass crucibles of medium porosity, and washing was performed with 95 per cent ethyl alcohol freshly saturated with sodium copper uranyl acetate. Moreover, the precipitate was dried at 60° C., not at 110° C. This was necessary because of its lower stability as compared to sodium magnesium uranyl acetate. The factor 0.0145 was used for converting the weight of dried precipitate into weight of sodium.

In Table V are shown results obtained with sodium present alone. It will be seen that by using a sufficient volume of reagent, amounts of sodium from 1 to 50 mg. may be determined with satisfactory accuracy. Experiments on the determination of quantities below 1 mg. indicated that 0.4 mg. is about the smallest quantity that may be successfully determined with this reagent when using ordinary volumes of reagent and sample solution.

In Table VI are shown results of determinations of sodium in the presence of lithium. Contrary to what was expected from the results of the qualitative experiments, the presence of lithium invariably caused a positive error in the sodium determinations. The magnitude of this error is apparently independent of the volume of reagent used, is decreased slightly by increasing the volume of sample solution, and is

TABLE VI. DETERMINATIONS OF SODIUM IN THE PRESENCE OF LITHIUM

Reagent	Sample Solution	Lithium Present	Sodium Present	Sodium Found	Difference
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
50	2	10.0	10.0	10.4	+0.4
		15.0	10.0	10.6	+0.6
		20.0	10.0	10.5	+0.5
		25.0	10.0	10.6	+0.6
100	2	10.0	10.0	10.5	+0.5
		15.0	10.0	10.8	+0.8
		20.0	10.0	10.6	+0.6
		25.0	10.0	10.7	+0.7
50	5	10.0	10.0	10.2	+0.2
		15.0	10.0	10.3	+0.3
		20.0	10.0	10.3	+0.3
		25.0	10.0	10.2	+0.2
		50.0	10.0	10.7	+0.7
100	5	10.0	10.0	10.3	+0.3
		15.0	10.0	10.6	+0.6
		20.0	10.0	10.4	+0.4
		25.0	10.0	10.6	+0.6
		50.0	10.0	11.1	+1.1
100	5	25.0	20.0	21.3	+1.3
		50.0	20.0	21.5	+1.5
		100.0	20.0	21.7	+1.7

TABLE VII. DETERMINATIONS OF SODIUM IN THE PRESENCE OF POTASSIUM

Reagent	Sample Solution	Potassium Present	Sodium Present	Sodium Found	Difference
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
50	2	50	10.0	10.2	+ 0.2
50	5	50	10.0	10.0	± 0.0
50	5	100	10.0	10.1	+ 0.1
50	5	200	10.0	10.2	+ 0.2
50	5	300	10.0	15.5	+ 5.5
100	2	50	10.0	10.4	+ 0.4
100	5	50	10.0	10.1	+ 0.1
100	5	100	10.0	10.4	+ 0.4
100	5	200	10.0	10.8	+ 0.8
100	5	300	10.0	20.6	+10.6
100	5	50	20.0	19.5	− 0.5
100	5	100	20.0	20.4	+ 0.4
100	5	200	20.0	21.1	+ 1.1
100	5	300	20.0	22.8	+ 2.8

increased with increase in amount of sodium precipitated. As shown also by the results in Table VI, the error is by no means proportional to the amount of lithium present. The errors in these determinations of sodium in the presence of lithium with the cupric acetate–uranyl acetate reagent are much smaller than the errors which would result in similar determinations with the magnesium acetate–uranyl acetate or the zinc acetate–uranyl acetate reagents. These errors are not serious when quantities of lithium up to 10 mg. are present and when suitable volumes of reagent and sample solution are employed. It seems likely from the present results that determinations of sodium in the presence of larger amounts of lithium could be accomplished with satisfactory accuracy if a suitable small correction were applied to the final results. Thus for purposes where high accuracy is not required, the alcoholic cupric acetate–uranyl acetate reagent may be useful for estimating sodium in the presence of both small and large amounts of lithium. In Table VII are shown results of determinations of sodium in the presence of potassium with this reagent. Interference from potassium is not serious unless considerable amounts are present, the extent of the interference being in the same order of magnitude as that from other sodium reagents of this type. The effect of ammonium is illustrated by the results in Table VIII. In contrast to effects observed with other sodium reagents, slightly low results are obtained with the alcoholic cupric acetate–uranyl acetate reagent when considerable amounts of ammonium are present. However, the interference from ammonium is not serious from the standpoint of practical analysis because excessive amounts are so readily removed.

From a manipulative viewpoint the use of an alcoholic cupric acetate–uranyl acetate for quantitative work is slightly less convenient than the use of aqueous magnesium acetate–uranyl acetate or zinc acetate–uranyl acetate reagents, since the crystals of sodium copper uranyl acetate are more difficult to transfer than those of the triple acetates precipitated by these other reagents by reason of their greater coarseness, density, and tendency to adhere to the walls of the vessel in which the precipitation is made. In general, the alcoholic cupric acetate–uranyl acetate reagent offers no advantages over other sodium reagents of this type unless determinations of sodium are to be made in the presence of lithium.

Other Possible Reagents

An apparently heretofore unexplored possibility by which the triple salt method for sodium might be improved is the use of metal salts of certain substituted acetic acids and of certain homologs of acetic acid in the preparation of sodium reagents. Reagents prepared from uranium and magnesium chloroacetates, formates, propionates, and butyrates, modeled after the magnesium acetate–uranyl acetate reagent of Caley and Foulk (*2*), were studied. The magnesium-uranyl salt type was selected for study because of the ease by which the magnesium salts of the acids could be prepared. The magnesium chloroacetates were prepared by the dissolution of both pure magnesium and pure magnesium carbonate in the pure acids; the formates, propionates, and butyrates by the dissolution of pure magnesium. The uranyl salts were prepared by the dissolution of pure uranium trioxide in the acids.

TABLE VIII. DETERMINATIONS OF SODIUM IN THE PRESENCE OF AMMONIUM

Reagent	Sample Solution	Ammonium Present	Sodium Present	Sodium Found	Difference
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
50	2	100	10.0	9.9	−0.1
50	5	100	10.0	9.8	−0.2
50	5	200	10.0	9.9	−0.1
50	5	300	10.0	9.9	−0.1
50	5	400	10.0	9.7	−0.3
100	2	100	10.0	10.0	±0.0
100	5	100	10.0	9.8	−0.2
100	5	200	10.0	9.7	−0.3
100	5	300	10.0	9.7	−0.3
100	5	400	10.0	9.4	−0.6

These reagents were tested qualitatively for sensitivity toward sodium, lithium, and potassium by adding 10 ml. of reagent to 1 ml. of chloride solutions of these ions. Surprisingly enough, not one of those possible reagents was sensitive toward sodium. No precipitate was obtained with the chloroacetate and formate reagents, even when highly concentrated sodium chloride solutions were tested. The propionate reagent yielded only a slight precipitate with 50 mg. of sodium, and although the butyrate reagent gave a large initial precipitate with the same amount of sodium, this precipitate redissolved before all the reagent was added. With lithium solutions the chloroacetate and formate reagents yielded no precipitates. The propionate reagent was somewhat more sensitive toward lithium than toward sodium, since a large precipitate was obtained with 50 mg. and a slight precipitate with as little as 20 mg. The butyrate reagent failed to precipitate even highly concentrated lithium solutions. With potassium solutions the chloroacetate and formate reagents also yielded no precipitates. The propionate reagent yielded a moderate precipitate with 50 mg. of potassium but not with 20 mg. On the other hand, the butyrate re-

agent produced a very heavy precipitate with 50 mg., a heavy precipitate with 20 mg., and a slight precipitate with 5 mg. but no precipitate with 1 mg. In other words, the butyrate reagent turned out to be a selective but not very sensitive reagent for potassium.

Contrary to what might reasonably have been expected, these reagents closely analogous to the acetate type of reagent were found to be entirely ineffective for the detection or determination of sodium.

Acknowledgment

Much of the work reported in this paper was done by Lockhart B. Rogers while holding a J. T. Baker Chemical Company Fellowship in Analytical Chemistry at Princeton University for the year 1941–42.

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Determination of Alkalies in Glass

Denatured Alcohols as Substitutes for U. S. P. Grain Alcohol

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THE procedures (*3*) for determining sodium and potassium in soda-lime glasses specify the use of 95 per cent ethyl alcohol in the preparation of wash solutions. In the method for sodium, the precipitate of sodium zinc uranyl acetate is washed with a saturated solution of the triple acetate salt in 95 per cent ethyl alcohol. In the potassium procedure the potassium chloroplatinate precipitate is washed with 80 per cent ethyl alcohol.

TABLE I. SODIUM OXIDE DETERMINATIONS ON GLASS

Alcohol Used	Na_2O, %
Alcohol denatured with 10% ether	16.81
	16.77
	16.81
Alcohol denatured with 10% acetone	16.79
	16.81
95% grain alcohol	16.80
	16.81
Bureau of Standards recommended value	16.83

The possibility of substituting one of the specially denatured alcohols for 95 per cent grain alcohol in the determination of sodium and potassium has been investigated. Two commercial denatured alcohols were selected for trial, one containing about 10 per cent of ethyl ether and the other about 10 per cent of acetone. These two products were compared with 95 per cent ethyl alcohol in a series of determinations of sodium and potassium in the National Bureau of Standards sample of soda-lime glass No. 128.

Sodium Determinations

Sodium (*3*) was precipitated as sodium zinc uranyl acetate and the precipitate was washed, first with the aqueous reagent, then with a solution of alcohol saturated with the triple acetate salt, and finally with ether. For purposes of comparison, quantities of the two selected denatured alcohols were saturated with the sodium salt and used in the same manner as the saturated wash solution prepared from 95 per cent grain alcohol.

Comparative results obtained on a series of 0.1-gram samples, Bureau of Standards glass No. 128, are given in Table I.

The percentages have been adjusted for the change in ignition loss on standard glass No. 128 occurring since the sample was distributed.

It is evident that the two denatured alcohols selected are satisfactory as substitutes for 95 per cent grain alcohol in the determination of sodium oxide in glass.

Potassium Determinations

Potassium (*3*) was determined by the modified Hicks method, in which the potassium is first separated as the chloroplatinate; this salt, after thorough washing with alcohol to eliminate soluble platinum compounds, is dissolved in hot water, then reduced to yield metallic platinum which can be separated and weighed. The original Hicks procedure (*2*) specifies a wash solution of ethyl alcohol of at least 80 per cent strength. For purposes of comparison, each of the denatured alcohols in question was diluted with 10 per cent water (by volume), resulting in a concentration of ethyl alcohol, exclusive of the denaturant, of approximately 80 per cent. These solutions were compared with 80 per cent ethyl alcohol in a series of K_2O determinations on Bureau of Standards glass No. 128 (Table II).

The question of optimum concentration of alcohol for washing potassium chloroplatinate precipitates has been discussed very recently by Ford and Hughes (*1*). In a series of potassium determinations on a large number of fertilizer samples, they found that the chloroplatinate precipitate is slightly more soluble in 80 per cent than in 95 per cent ethyl alcohol. They conclude that undiluted 95 per cent alcohol is a little more satisfactory than the diluted form as a wash solution for potassium chloroplatinate.

The tests indicate that the two denatured alcohols in question may be used satisfactorily to replace 95 per cent grain alcohol in the potassium procedure.

TABLE II. POTASSIUM OXIDE DETERMINATIONS ON GLASS

Wash Solution	K_2O, %
90 parts of ether-denatured alcohol + 10 parts of water	0.98
	0.99
90 parts of acetone-denatured alcohol + 10 parts of water	0.95
	0.97
80 parts of 95% alcohol + 20 parts of water	0.99
	0.97
Bureau of Standards recommended value	0.99

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Spray Residues of Tartar Emetic on Citrus Leaves

Determination by Iodometric Titration

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TARTAR emetic is now used to control certain species of thrips, especially the citrus thrips, *Scirtothrips citri* (Moult.), on lemons and oranges. When it is used for the control of the citrus thrips, it is combined with sucrose and applied with a broom gun or a high-speed blower sprayer. During the 1940 season in California a spray solution was used containing 1.5 pounds (680 grams) of tartar emetic, 2 pounds (900 grams) of sucrose, and 100 gallons (378 liters) of water. Davidson, Pulley, and Cassil (*1*) have proposed a modified Gutzeit method for the determination of tartar emetic spray residues. However, it was desired to develop a simpler method that would eliminate ashing of the leaf material.

Table I. Tartar Emetic Deposits on Orange Foliage

(Sprayed with 1.5 pounds of tartar emetic and 2 pounds of sugar in 100 gallons)

Time from Spraying to Sampling *Days*	No. of Whole Leaves Taken	Deposit[a] *Micrograms/sq. cm.*
	Plot 1 at Redlands	
0	50	9.4
	50	7.8
	48	10.3
	50	6.6
	49	10.7
	50	7.0
Rain		
13	50	2.6
	50	3.0
	50	1.9
	51	1.5
21	25	1.9
	25	1.3
	27	1.5
	24	1.2
	Plot 2 at Redlands	
7	25	4.5
	25	5.1
	25	6.7
	25	6.4
	Plot 1 at San Bernardino	
0	49	9.2
	50	7.4
Rain		
13	47	1.5
	50	1.1

[a] A correction factor, 1.80 micrograms per sq. cm. for leaf material.

Direct titration with iodine solution in the presence of sodium bicarbonate is a recognized means of determining antimony in solution in the trivalent form in which it exists in tartar emetic [$K(SbO)C_4H_4O_6.{}^1/_2H_2O$]. Preliminary experiments showed that this titration was not significantly altered by the presence of sucrose, or by tartaric acid, which it was proposed to use as an aid to the removal of the spray from the leaves. Additional experiments showed that known amounts of tartar emetic applied to leaves could be quantitatively recovered by immediate washing with 1 per cent tartaric acid solution. If the treated leaves were allowed to dry overnight, or were kept in a moist chamber for several days, the titer was greater than the tartar emetic applied, but when corrected for the leaf material the final recoveries averaged about 98 per cent.

For example, 3.07 ml. of solution containing 5.78 mg. of tartar emetic were added to the surface of 13 leaves and allowed to dry overnight. The following morning the residue was removed with tartaric acid and titrated with iodine; 5.84 mg. of tartar emetic were removed from the leaves compared with 5.78 mg. added. The correction factor for the leaves used was 0.02 mg. per 32.6 sq. cm. of leaf surface (average area of leaf). When the correction factor was applied for the leaf material (0.26) the actual amount removed was 5.58 mg. compared with 5.78 mg. added. In another test the leaves were allowed to stand for 3 days in a standard moisture cage (*2*). When they were removed, washed with tartaric acid, and titrated with iodine, 8.82 mg. of tartar emetic were found compared with 8.51 mg. added. When the above figure was corrected (0.02 × 17 leaves) for the leaf material, 8.82 mg. − 0.34 mg. = 8.48 mg.

The use of the method is not valid in the presence of arsenites, but these compounds are seldom, if ever, applied to citrus trees.

Procedure

Replicate samples are collected from a plot of trees sprayed with tartar emetic by selecting at random a few leaves from each of several trees until approximately 25 or 50 have been taken. The replicate samples need not come from the same trees. Similar samples are collected from unsprayed trees. The leaf material is placed immediately in a portable ice box and taken to the laboratory, where it is stored in a refrigerator until analyzed. (Samples have been satisfactorily kept for 18 days.) As soon as practicable the leaves are washed with water containing 1 per cent of tartaric acid, first by agitation in a container and then individually by means of a wash bottle. The washings are made to 250 ml. for a 25-leaf sample and to 500 ml. for one of 50 leaves.

Aliquants of 100 ml. are neutralized with solid sodium bicarbonate and enough is added to saturate the solution. It is immediately titrated with standard approximately 0.01 *N* iodine solution, using freshly prepared starch solution as indicator. The iodine must be added at a uniform rate (approximately 10 seconds per ml.).

After the leaves have been washed, their outlines are traced on paper and the area is determined with a planimeter. The results in this paper are reported in terms of residue per square centimeter of leaf, and not on the basis of total leaf surface.

The correction to be applied is determined by carrying the unsprayed samples through the same procedure.

For residues on lemons approximately 15 fruits are taken as a sample. They are assumed to be prolate spheroids, and the area is calculated from measurements of the major and minor axes.

That the titration obtained is really due to antimony is confirmed by Reinsch's test, in which a strip of bright copper is boiled for 5 minutes in a portion of the solution to which has been added one sixth its volume of concentrated hydrochloric acid. A black or violet lustrous deposit is obtained.

Table II. Tartar Emetic Deposits on Lemon Foliage and Lemons

(At San Fernando, Calif., 12 days after spraying with 1.5 pounds of tartar emetic and 2 pounds of sugar in 100 gallons)

Sprayed Material Analyzed	No.	Deposit *Micrograms/sq. cm.*
Leaves[a]	51	1.6
	49	2.4
Lemons[a]	14	0.9
	14	0.7

[a] A correction factor of 1.50 micrograms per sq. cm. was used for leaves and 1.60 micrograms per sq. cm. for lemons.

Results

With this technique tartar emetic deposits have been determined on citrus foliage and on lemons from groves in several sections of southern California. The results are shown in Tables I and II.

Summary

The antimony residues from spraying citrus trees with tartar emetic and sugar can be removed from the leaves by washing with dilute tartaric acid solution and determined by titrating with standard iodine solution. The residues from orange foliage from a spray containing 1.5 pounds of tartar emetic and 2 pounds of sugar per 100 gallons were found to contain antimony equivalent to 6.6 to 10.7 micrograms of tartar emetic per square centimeter immediately after spraying, but these residues were greatly reduced by rain.

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Determining an Alkali Carbonate in the Presence of an Alkali Bicarbonate

A Colorimetric Method

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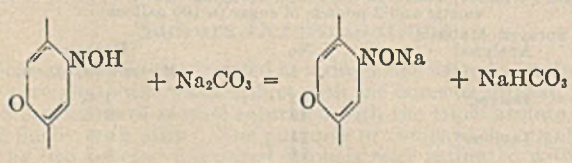
THE carbonates and bicarbonates of the alkali metals are important industrial chemicals and useful chemical reagents, since they are the salts of a strong base and a weak acid and are the only readily available salts of these anions which are soluble in water.

It is frequently necessary to distinguish between the carbonate and bicarbonate of an alkali metal, and to determine one in the presence of the other, since solutions of bicarbonates lose carbon dioxide to revert to the corresponding carbonate and solutions of the carbonates absorb carbon dioxide to become contaminated with the bicarbonate.

The pH of an alkali carbonate solution is higher than that of the corresponding bicarbonate; hence, these anions may be distinguished by their behavior with an indicator such as turmeric (*7*), which is reddened by solutions of an alkali carbonate but not by solutions of an alkali bicarbonate. Mercuric chloride (*6*), which gives a brownish-red precipitate with solutions of an alkali carbonate and a white precipitate with solutions of the bicarbonate, has been used to distinguish between these anions; as has magnesium sulfate (*5*), which precipitates at room temperature with alkali carbonates but not with the corresponding bicarbonates.

Qualitative Procedure

In a study of the tautomerism of *p*-nitrosothymol and thymoquinone monoxime (*9*) it was observed that a solution of sodium carbonate was alkaline enough to tautomerize the colorless *p*-nitrosothymol into thymoquinone monoxime with the simultaneous production of a red color due to the presence of the anion of the sodium salt of the oxime:



A solution of sodium bicarbonate under the same conditions produces no color or a very faint yellow color, depending on the amount of carbonate contamination in the bicarbonate sample. Thus *p*-nitrosothymol can be used to distinguish between alkali carbonates and bicarbonates and provides a qualitative test for the presence of an alkali carbonate in a sample of an alkali bicarbonate.

p-Nitrosothymol is available from the Eastman Kodak Company, Rochester, N. Y. It can also be prepared in almost quantitative yields by the method of Kremers and Wakeman (*3*). Purification of *p*-nitrosothymol can be accomplished by recrystallization from benzene or from diluted alcohol with the use of activated carbon.

Quantitative Procedure

There are standard procedures (*10*) for titrating an alkali carbonate in the presence of an alkali bicarbonate by the use of selected indicators. The accuracy of these methods depends upon several factors, especially the choice of the indicator, but under no condition is it exact when the amount of bicarbonate is proportionately large (*2*).

For determining inadmissible amounts of carbonate in official samples of the alkali bicarbonates, the U. S. Pharmacopoeia XI (*11*) requires that a 1-gram sample of the salt be not alkaline to phenolphthalein after it has been dissolved in 20 ml. of distilled water below 15° C. and treated with 2 ml. of 0.1 *N* hydrochloric acid. The British Pharmacopoeial method (*1*) is similar, except that thymol blue is used as the indicator. While these pharmacopeial methods serve the purpose for which they are intended, they are not quantitative.

The qualitative test using *p*-nitrosothymol was investigated to determine whether the intensity of the color produced with the indicator was in direct ratio to the amount of alkali carbonate present, so that it could be used for quantitatively determining an alkali carbonate in the presence of the corresponding bicarbonate.

Experimental

To a series of Nessler tubes, arranged in a rack fitted with a white porcelain base, were added equal volumes of sodium carbonate solutions of graduated molarity. To each tube was then added approximately twice the calculated quantity of *p*-nitrosothymol previously dissolved in enough neutral acetone or neutral dioxane to give a 0.35 *M* solution. (The *p*-nitrosothymol was dissolved in the solvent to facilitate its admixture with the carbonate solutions.) The tubes were shaken for from 10 to 15 minutes, and the excess *p*-nitrosothymol was filtered off. These filtrates provide the reference standards. An equal volume of a solution containing an unknown amount of alkali carbonate was treated in like manner, after which its intensity of color was compared to those of the reference standards for the determination of its alkali carbonate content.

By this procedure, using a blank composed of *p*-nitrosothymol and distilled water, it was found that the color produced by the indicator in the presence of 0.0001 *M* solutions of sodium carbonate could be detected with the unaided eye. From this molarity the method is applicable in concentrations up to 0.1 *M* sodium carbonate, above which the color is too

intense for comparisons. Near the lower concentration it is possible to distinguish between a 0.0001 and a 0.00015 *M* sodium carbonate solution, and at the higher concentration between a 0.1 and 0.09 *M* solution.

Three different procedures were used to check the accuracy of the *p*-nitrosothymol method against other methods used to determine alkali carbonates in the presence of alkali bicarbonates.

A solution of sodium carbonate of known molarity was prepared by dissolving reagent monohydrated sodium carbonate in distilled water. Measured portions of this solution were assayed by titration with standardized hydrochloric acid, using methyl orange as an indicator. Identical samples were assayed by the use of *p*-nitrosothymol in the manner described above. Twelve color reference standards ranging from 0.01075 to 0.01625 *M* with respect to sodium carbonate were used, and each successive tube in the series differed to the extent of 0.0005 in molarity. The results by the two methods are:

Molarity of solution with respect to Na_2CO_3	
True molarity	0.01325
As determined with HCl (average of 3 determinations)	0.01338
As determined with *p*-nitrosothymol (average of 5 readings)	0.01340

A 0.15 *M* solution of sodium bicarbonate was treated with carbon dioxide until it no longer gave a color with *p*-nitrosothymol. To this solution were added enough sodium carbonate and distilled water to give a solution which was 0.075 *M* with respect to sodium bicarbonate and 0.00525 *M* with respect to sodium carbonate. The amount of sodium carbonate in this solution was then determined by Kuster's method (*4*), which consists of titrating the solution at 0° C. in the presence of an alkali chloride with standardized acid, using phenolphthalein as the indicator. The amount of sodium carbonate was determined also with the use of *p*-nitrosothymol in the manner described above. Twelve color reference standards which ranged from 0.00275 to 0.00825 *M* with respect to sodium carbonate were used, and each successive tube in the series differed to the extent of 0.0005 in molarity. The results by both methods are:

Molarity of solution with respect to Na_2CO_3	
True molarity	0.00525
As determined with HCl (average of 3 determinations)	0.00735
As determined with *p*-nitrosothymol (average of 5 readings)	0.00612

The amount of sodium carbonate in a sample of sodium bicarbonate was determined with *p*-nitrosothymol in the manner previously described. To an approximately 0.15 *M* solution of this sodium bicarbonate were added enough 0.0175 *M* sodium carbonate solution and distilled water to give a solution which was approximately 0.075 *M* with respect to sodium bicarbonate and 0.00575 *M* with respect to sodium carbonate. The sodium carbonate in aliquot portions of this solution was determined by titration with standardized hydrochloric acid, using a mixed indicator composed of thymol blue and cresol red as recommended by Simpson (*8*). Other aliquot portions were assayed for sodium carbonate, using *p*-nitrosothymol in the manner described above. Twelve color reference standards which ranged from 0.00275 to 0.00825 *M* with respect to sodium carbonate were used, and each successive tube in the series differed to the extent of 0.0005 in molarity. The results by the two methods are:

Molarity of solution with respect to Na_2CO_3	
True molarity	0.00575
As determined with HCl (average of 3 determinations)	0.00620
As determined with *p*-nitrosothymol (average of 5 readings)	0.00600

Stability of Reference Standards

The influence of aging of the reference standards on the accuracy of the method was determined.

A series of twelve reference standards which ranged from 0.00275 to 0.00825 *M* with respect to sodium carbonate, with each successive tube differing to the extent of 0.0005 in molarity, was prepared by the method described above, except that the indicator was shaken with the known carbonate solutions for 30 minutes. The reference standards were kept for 8 days, during which they were checked periodically with a carbonate solution of known molarity which was prepared just prior to the periodic comparisons by the same procedure used with the reference standards.

The results of these comparisons are:

True molarity of solution with respect to Na_2CO_3	0.00525
Molarity with respect to Na_2CO_3	
Determined with fresh standard (average of 3 determinations)	0.00537
Determined with day-old standard (average of 3 determinations)	0.00550
Determined with 3-day-old standard (average of 3 determinations)	0.00565
Determined with 5-day-old standard (average of 3 determinations)	0.00567
Determined with 8-day-old standard (average of 3 determinations)	0.00567

Discussion

While all the foregoing experiments involved the use of only sodium carbonate and sodium bicarbonate, it was found by preliminary experiments that the method could be applied to the corresponding salts of lithium and potassium.

For the *p*-nitrosothymol method to be accurate and its results reproducible, the conditions for mixing the bicarbonate solution must be kept the same as those under which the reference standards are prepared from known alkali carbonate solutions and *p*-nitrosothymol.

There was no difference in the intensity of the color produced when 1.1 or 2 times the calculated quantity of *p*-nitrosothymol was added to the alkali carbonate solutions. Addition of approximately twice the calculated quantity of *p*-nitrosothymol provides a safe margin, especially in the case of the unknowns where the carbonate content would have to be estimated.

As may be seen from the experimental data, the method is more accurate when freshly prepared reference standards are used. The reference standards change less when they are kept in a closed cupboard than when they are exposed to the light.

As was expected, boiling a solution of sodium carbonate or sodium bicarbonate with the indicator gave a darker color than when solutions of the same strength were shaken with the indicator at room temperature.

The *p*-nitrosothymol method is most accurate when only a little carbonate is present with much bicarbonate, the conditions under which the titration method is least exact.

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A Schematic Procedure for Identification of Common Commercial Plastics

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THE use of synthetic resins or plastics is daily becoming more and more important. Not only are these materials being used for original purposes, but, even more important, they are gradually replacing tremendous quantities of metals and other vital materials in war industry.

The chemist, especially in the commercial or industrial testing laboratory, has to keep up with the great strides taken by the plastics industry if he is to remain valuable in his position. Tables of the properties and characteristics of the synthetic resins are readily available (*3*) but, to the best of the author's knowledge, no method has as yet been published to aid the chemist in the identification of a particular plastic.

This article presents, in schematic form, a simple and concise procedure for the identification of the more commonly encountered synthetic resins or plastics. Not all the known plastics are considered in this procedure, but only those which are rather uncommon under ordinary circumstances have been omitted. Only those materials which one is likely to find in consumer merchandise submitted for test have been included. Naturally, with the development of new materials and the appearance of new plastics on the commercial market, additions and revisions of the method will become necessary.

A novel feature of the procedure is the rather wide application of the olfactory sense. In most cases the odors are so intense that rather cautious smelling is advised.

An important effect to be noted in the classification is the "green zone" around the base of the flame. This green color, when present, will be found at the area closest to the burning plastic and will be readily discernible. In all the burning tests a strip or small rod, if practicable, should be touched to the side of a small flame. If it takes fire readily, it should be removed from the flame at once; if it melts and draws away from the flame, it should be moved so that it is kept in the flame until it takes fire, but not for more than 10 seconds. When the flame of the plastic is to be examined after removal from the Bunsen flame, this should be done immediately following the removal, disregarding any burning characteristics occurring after the first 2 seconds.

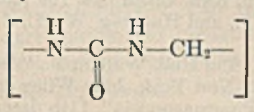
To determine the odor of vapors produced, the flame should be blown out immediately upon removal from the Bunsen flame and the rising vapors should be smelled cautiously. No valid conclusions can be reached from smelling the plastic while it burns, and too much vapor will produce a deadening effect upon the olfactory organs.

In all cases it is advisable to have specimens of the various plastics in the laboratory, for comparison of the tentatively identified unknown material with a known specimen. The ideal forms in which plastics are to be examined are sheets and rods.

Nomenclature

UREA FORMALDEHYDE, the thermosetting polymer produced by the condensation of urea and formaldehyde, is usually glassy and infusible. Heating causes the resin to blacken and it ignites only with extreme difficulty.

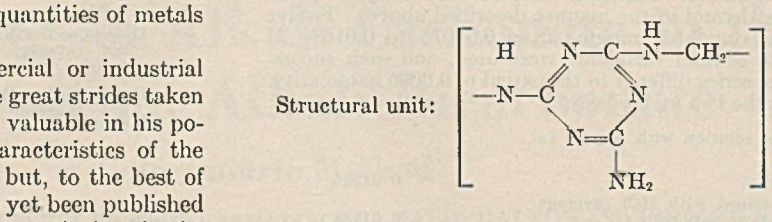
Structural unit:



Represented by Beetleware and Plaskon

MELAMINE FORMALDEHYDE, similar to urea formaldehyde in properties and appearance, is formed by the condensation of melamine and formaldehyde.

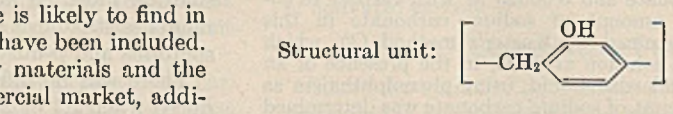
Structural unit:



Represented by Melamac and Plaskon

PHENOL FORMALDEHYDE, the thermosetting resin formed by the condensation of phenol and formaldehyde, is one of the most widely used of all plastics. The original work of Baekeland (*1*) describes the variation of the properties of the polymer with methods of preparation and processing.

Structural unit:

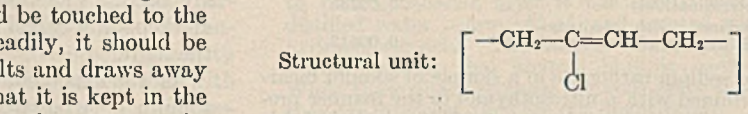


Represented by Durite and Bakelite

PLIOFILM, a thermoplastic resin produced by the addition of hydrogen chloride to the unsaturated linkage present in rubber. It is widely used in sheet form as a protective covering for various articles.

NEOPRENE, a rubberlike synthetic material produced by polymerization of the addition product of vinyl acetylene and hydrogen chloride. It has properties remarkably superior to those of the natural product it can replace, rubber.

Structural unit:



VINYL CHLORIDE DERIVATIVES, thermoplastic resins produced by polymerization of vinyl chloride with itself or with other compounds such as vinyl acetate. The products are elastic and may be transparent. Many of the plastic suspenders and wristwatch bands are made of this material.

Represented by Vinylite V and Koroseal

CASEIN. These are infusible resins produced by the processing and molding of milk protein. The products are usually opaque, but may clear if the casein is hydrolyzed first. The material is very tough.

CELLULOSE ACETATE, a thermoplastic product produced by the reaction between acetic anhydride and cellulose, is an ester of the alcohol cellulose. Safety film is prepared from this material.

Represented by Lumarith, Tenite I, and Fibestos

CELLULOSE NITRATE, the resin produced by the nitration of cellulose with mixed sulfuric and nitric acids. When plasticized with camphor it is known as celluloid. It is a highly flammable material which is finding less and less use with the development of newer plastics because of this hazard.

Represented by Celluloid and Hycoloid

METHACRYLATE RESINS, thermoplastic products formed by polymerization of one or more compounds of the type α-methyl methacrylate, methyl acrylate, ethyl methacrylate, etc. The product is readily prepared clear and colorless. The aviation industry is becoming one of the chief users of this resin. A disadvantage of the material is its comparatively low resistance to abrasive materials.

Represented by Plexiglas and Lucite

POLYVINYL FORMAL, ACETAL, AND BUTYRAL, thermoplastic resins produced by the condensation of polyvinyl alcohol with formaldehyde, acetaldehyde, and butyraldehyde, respectively. They are not very frequently encountered in consumer goods, but

show definite promise of much wider application than they enjoy at present.

Represented by Formvar, Alvar, and Butvar

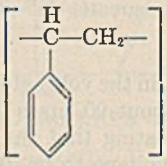
NYLON, the condensation polymer formed by the interaction of adipic acid and hexamethylenediamine. It is thermoplastic and has exceptional tensile strength. Nylon stockings, bristles, and tennis racket strings on the market illustrate the versatility of this product. Parachute manufacture consumes most of our present output.

Structural unit: $\left[-C-(CH_2)_4-C-(CH_2)_6-NH-\right]$ (O above and below the carbonyl carbons)

CELLULOSE ACETOBUTYRATE, a thermoplastic resin produced by reacting cellulose with acetic butyric anhydride, $C_3H_7CO-O-OCCH_3$. It may be prepared in transparent sheets or in heavy opaque form. Like cellulose acetate, this product is an ester of cellulose.

Represented by Tenite II

POLYSTYRENE, a thermoplastic material produced by polymerization of the aromatic hydrocarbon, styrene. It is therefore highly resistant to attack by inorganic acids and alkalies. It is very widely used.

Structural unit: 

Represented by Styron, Lustron, and Loalin

ETHYL CELLULOSE, an ether formed by the intermolecular dehydration of cellulose and ethyl alcohol. It is very similar in appearance and properties to the ester cellulose acetate.

Represented by Ethocel

CELLULOSE, the common transparent material widely used for wrapping packages. Strictly speaking it is not a synthetic resin, but merely reprecipitated cellulose. It is included, however, because of its close resemblance to other materials used for similar purposes.

Represented by Cellophane

VINYLIDENE CHLORIDE RESINS, the newly developed very resistant resins produced by the polymerization of vinylidene chloride, unsymmetrical dichloroethylene. It is produced in filament and sheet as well as tube form.

Structural unit: $[-CCl_2-CH_2-]$

Represented by Saran and Velon

Procedure

The plastic, in strip or rod form, if possible, is held at one side of a Bunsen flame until it takes fire, but not for more than 10 seconds. The following scheme is then consulted:

I. No flame is produced. The article retains its shape. The odor of formaldehyde is present in all cases.
   A. No other odor: urea formaldehyde
   B. Strong fishlike odor: melamine formaldehyde
   C. Phenolic odor: phenol formaldehyde

II. The burning plastic extinguishes itself on removal from the Bunsen flame. Specimen held just to edge of flame.
   A. A green zone is produced.
      1. Odor of burnt rubber
         a. Green area pronounced: pliofilm
         b. Green area small and overshadowed by yellow: neoprene
      2. Odor acrid but not that of burnt rubber: vinyl chloride derivative
      3. Odor sweet, heavy black ash: vinylidene chloride resins
   B. Odor of burnt milk protein: casein
   C. Sparks from flame of burning plastic and acetic odor: cellulose acetate

III. The plastic continues to burn after removal from the Bunsen flame. Flame examined for color during the first second of burning.
   A. Burning very rapid and intense white flame
      1. Odor of camphor: celluloid
      2. No camphor odor: cellulose nitrate
   B. Flame predominantly blue, may have a small white tip
      1. Very strong sweet floral fruity odor: methacrylate
      2. Odor reminiscent of burning vegetation or fresh celery. Flame is almost entirely blue. The resin is soluble in aqueous 60 per cent (by volume) hydrochloric acid (*2*): nylon
      3. Weak very slightly sweet odor: polyvinyl formal
      4. Odor of rancid butter or cheese
         a. Sparks produced from flame of burning plastic: cellulose acetobutyrate
         b. No sparks, burning smooth and steady: polyvinyl butyral
   C. Flame surrounded by bright green mantle, odor of burnt rubber: pliofilm
   D. Flame surrounded by a purple mantle, sparks and an acetic odor: polyvinyl acetal
   E. Flame yellow-white and luminous
      1. Odor of butyric acid: cellulose acetobutyrate
      2. Odor of burnt milk protein: casein
      3. Sweet floral odor (marigold), flame smoky: polystyrene
      4. Weak very slightly sweet odor: polyvinyl formal
      5. Odor of burnt paper: cellulose
      6. Odor of burnt rubber, flame weak, small green mantle overshadowed by yellow. White flashes may appear in the flame: neoprene
   F. Flame surrounded by yellow-green mantle
      1. Burns with difficulty and sparks, producing an acetic odor. The melted burning plastic, when allowed to drip into water, produces heavy black-brown foamy granules or flakes: cellulose acetate
      2. Burns readily, once started, producing a slight sweet odor. The melted burning plastic, when allowed to drip into water, produces flat disks which are light tan when the plastic is clear, or not very different in color from the original plastic when the plastic is colored: ethyl cellulose

The same resin may occupy more than one position in the scheme. This is to take into account any possible misinterpretations of the characteristics described.

Summary

A schematic procedure for identification of the commercial plastic types includes the plastics which the average chemist may encounter in his testing experiences. Seventeen families of plastics are discussed briefly, representing about one hundred plastics which are marketed under different commercial names.

Acknowledgment

The author wishes to acknowledge the valuable assistance given him by the Dow Chemical Company, the Carbide and Carbon Chemicals Corporation, and the Celluloid Corporation in sending him some of the specimens used as standards in the evolution of the method.

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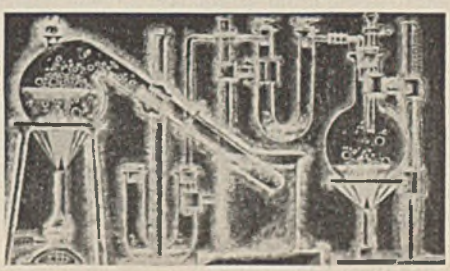

A Versatile Continuous Reading Thermionic Voltmeter

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THERMIONIC instruments for potential measurement can be roughly divided into two classes: vacuum tube voltmeters for voltage measurements in low-resistance circuits and electrometers for voltage measurements in high-resistance circuits. Few attempts have been made to combine into one instrument those characteristics necessary for high-resistance work with desirable features usually found only in vacuum tube voltmeters. The increasing use of the glass electrode and the phototube have put a premium on the development of such universal instruments.

A satisfactory laboratory voltmeter for all-purpose work should be designed with the following characteristics in mind:

Necessary	Desirable	
Low grid current	Continuous reading	Variable sensitivity
Stability	Simplicity of operation	Circuit simplicity
High sensitivity	Wide operating range	Low cost of construction
	Low drift rate	Portability

All these characteristics are not entirely compatible. The great majority of potential measuring circuits having the "necessary" characteristics have been designed around special electrometer tubes of relatively high cost or are circuits suitable only for use as null-point indicators (*4, 6, 8, 11, 13, 14, 15, 18, 22, 28, 29*). None of these instruments has enough of those characteristics listed as desirable to make it suitable for general laboratory use.

For potentiometric titration work an instrument that continuously indicates the potential is most desirable, since it allows the course of the equilibrium to be followed at all times. While several continuous-reading titrimeters have been described (*10, 12, 25, 32, 33*), few continuous-reading voltmeters suitable for high-resistance work are to be found in the literature (*16, 24, 31*). Of the circuits published, that of Penther, Rolfson, and Lykken (*24*) is the most promising for work in high-resistance systems. The principal limitation of their instrument is that it has a range of linear response of only 0.110 volt. Further voltage changes must be compensated for with a step-potentiometer. In titrations of a routine nature where speed is important, it would be more advantageous to have an instrument with a wider voltage range requiring no adjustment during the course of the titration.

The circuit described below was developed in an attempt to combine in one instrument as many of the listed features as possible.

Circuit Synthesis

Recently Garman and Droz (*10*) designed a simple vacuum tube voltmeter employing the bridge-type circuit usually found in stable electrometers. The tube used in their circuit is an ordinary pentagrid converter, type 1A7GT, which has the necessary characteristics for an electrometer tube. The 1A7GT is particularly useful, since it also has a low filament current, with resulting low heat emission and battery drain.

In the circuit of Garman and Droz the plate and anode grid of the pentagrid converter are connected as a conventional Wheatstone bridge. The voltage to be measured is impressed between the control grid and filament and results in a simultaneous increase in plate current and an equal decrease in anode grid current or vice versa. This results in doubly unbalancing the bridge. In their original circuit Garman and Droz connect a sensitive current meter across the bridge to indicate the degree of unbalance. A high-resistance meter would be desirable, to build up a maximal potential difference across the bridge, but a low-resistance meter would give a greater deflection for a given voltage difference.

Experiment showed that, in the voltmeter described, increasing the meter resistance about 90 times decreased the sensitivity only threefold, indicating that the high resistance allowed 30 times as high a voltage to be developed across the bridge as the low resistance. The ideal meter, then, should have a very high resistance as well as a high sensitivity.

The ideal meter characteristics are approached by connecting across the bridge of the Garman-Droz circuit an ordinary vacuum tube voltmeter (Figure 1). The second stage is, in effect, a volt-

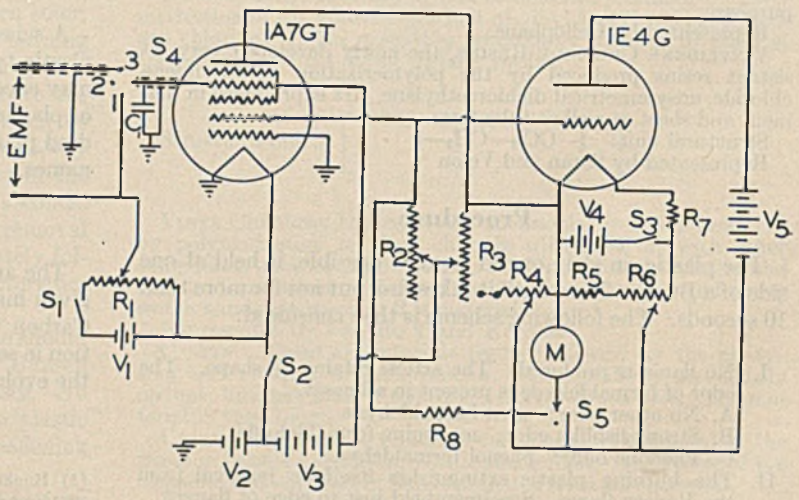

FIGURE 1. VOLTMETER CIRCUIT

C_1. Tested condenser, capacity 0.02 mfd.
M. 0–500 (120-ohm internal resistance) or 0–100 (900-ohm internal resistance) Triplett microammeter
R_1. 10,000-ohm uniform volume control
R_2. 5000-ohm uniform volume control
R_3. 10,000-ohm uniform volume control
R_4. 2000-ohm uniform volume control (for use with 0–500 microammeter) with switch cover plate for S_3
R_5. 5000-ohm 0.5-watt
R_6. 5000-ohm tapered volume control with switch cover plate for S_1 and S_2
R_7. 30-ohm 0.5-watt
R_8. 2000-ohm 0.5 watt
S_1, S_2. Double-pole single-throw switch
S_3. Single-pole single-throw switch
S_4. Special high-resistance SP3 position switch with off position in center
S_5. SP3 position switch with off position in center
V_1. 2 1.5-volt flashlight cells
V_2. 1.5-volt filament battery
V_3. 45-volt plate battery
V_4. 2 1.5-volt filament batteries
V_5. 2 45-volt plate batteries

[1] Present address, U. S. Navy Radio and Sound Laboratory, San Diego, Calif.

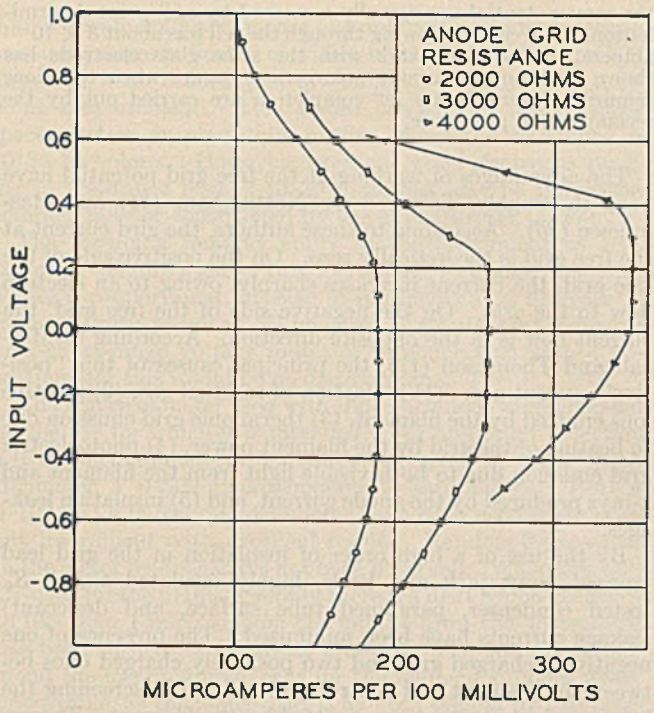

FIGURE 2. SENSITIVITY VARIATION WITH BRIDGE RESISTANCE

meter having a resistance of several megohms but with a sensitivity of about 850 microamperes per volt. The 1E4G tube was chosen for this stage because it has advantages over other voltage amplifiers in possessing a low filament current, high transconductance, zero grid bias, and the high stability inherent in triodes.

To this basic circuit were added several other features for increasing the versatility of the instrument. One of these is the potentiometer circuit, R_1-V_1, in the *EMF* input lead to the filament of the electrometer tube.

The bridge resistances, R_2 and R_3, are separate variable resistances to permit varying the instrument sensitivity as desired (see Figure 2). The top view of the instrument (Figure 3) shows the relative positions of R_2 and R_3. For operation at a given maximum sensitivity, either the plate resistance, R_3, or the anode grid resistance, R_2, may be fixed. S_5 and R_8 are included in order that the microammeter may be used for balancing the bridge.

Stadie, O'Brien, and Laug (*30*) point out the desirability of increasing the capacity of the control grid by connecting to it a suitable grounded condenser, C_1. This increased capacity serves to protect the grid potential from momentary changes due to the opening of switch S_4. In order to prevent leakage currents, this condenser must have a resistance at least as high as the internal resistance of the control grid. To reduce leakage further it is necessary to construct S_4 and the input jack in the grid lead with a high degree of insulation from ground. Many suitable switch designs have been published (*6, 13, 28, 30*). The authors have found that glass tubing makes satisfactory stand-off insulators for this purpose. These can be fastened to the chassis by means of a short bolt and de Khotinsky cement. The jack in the grid circuit was designed to take the Beckman glass electrode fittings. The electrometer tube is shielded by means of a glass cylinder fitted over the tube base and covered with a metal cap connected to ground. The shielded lead to the grid of the electrometer tube passes through this metal cap (Figure 3). The surface of the 1A7GT and the glass cylinder are covered with paraffin to prevent leakage of surface currents. The control grid lead is the only part of the circuit where extraordinary care with regard to insulation need be taken.

In the second stage there are two necessary controls. One of these, R_4, varies the over-all sensitivity by shunting the microammeter. A value of R_4 equal to about 15 times the meter resistance gives a good spread of the sensitivity control with only 6 per cent reduction in maximum sensitivity due to shunting. This reduction can also be eliminated if contact is broken at the extreme end of the shunt. Coating the end of the contact surface with an insulating material is a convenient way to do this. The other control, R_6, is in the widely used D-battery circuit (*9, 26*), which is composed of R_5 and R_6 and the 6-volt filament battery. For a given setting of R_6 a fixed amount of plate current is bypassed around the meter, thus allowing the use of a more sensitive meter. The resistance of both R_5 and R_6 should be kept high to reduce battery drain and to increase the by-passing range. Under these conditions, a small rotation of R_6 causes a large change in the amount of current by-passed. For ease of control a vernier is desirable. An ordinary planetary control with a 5 to 1 ratio has been found satisfactory. The combination of the filament and D-batteries is a commonly used method of stabilizing the circuit against changes in battery voltages (*16, 19, 25, 31*).

The entire instrument with all batteries is enclosed in a 7 × 8 × 10 inch metal cabinet. Figures 3 and 4 show the panel layout and some details of the internal arrangement.

Operation and Adjustment

The preliminary steps in the operation of the instrument are: (1) S_4 is opened (position 2, Figure 1); (2) S_1 and S_2 are closed by rotating R_6 and the rotation is continued to about the center of the range; (3) S_3 is closed by rotating R_4.

In the preliminary adjustment of the instrument it is necessary at this point to balance the bridge by varying R_2 or R_3. At least an hour should be allowed for the electrometer tube to reach equilibrium before this adjustment is made. Switch S_4 is opened and then the microammeter in the second stage is temporarily connected across the bridge by closing S_5. The plate resistance, R_3, is fixed at a convenient value and the anode grid resistance, R_2, is adjusted until the current through the meter is zero. This adjustment need only be checked occasionally after the tube has sufficiently aged. When the adjustment has been made the meter is again switched back into the second stage.

Finally, the free-grid potential is located as follows: (1) With the sensitivity considerably reduced, S_4 is connected to the internal biasing battery (position 1, Figure 1). (2) The bias is adjusted by rotating R_1 until the meter gives the same reading as with the grid floating. The sensitivity is now increased and R_1 further adjusted until no drift or deflection occurs in the meter on opening and closing S_4 to the internal bias. The needle is always kept on the scale by means of R_6.

With these preliminary adjustments completed, the instrument is ready for use. The subsequent procedure depends on the type of measurement to be made.

For potentiometric titrations with low-resistance electrodes the connection of the electrodes is immaterial. The initial electrode potential is set to the free grid with the biasing control. The needle is set either to the upper or lower end of the meter scale with the D-battery control, depending upon the direction of voltage change expected during the titration. The circuit is so connected that the microammeter needle is deflected downscale as the control grid becomes more negative.

When high-resistance electrodes such as the glass electrode are used, they are always connected to the grid lead, since one side

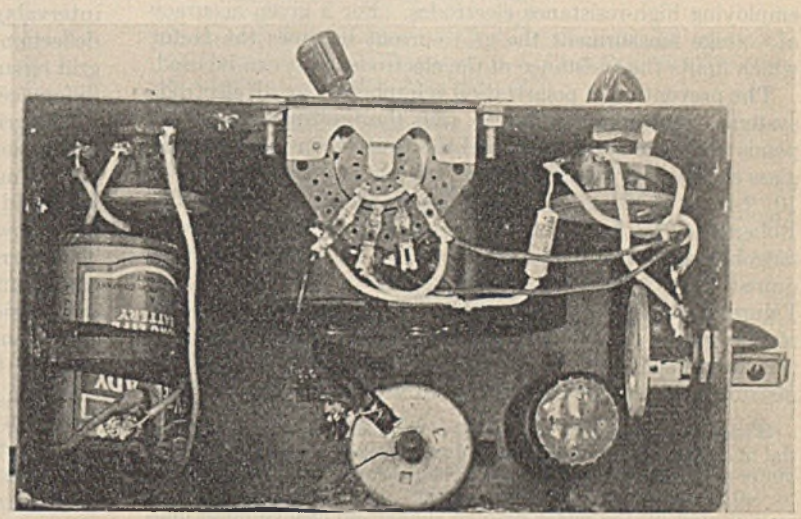

FIGURE 3. TOP VIEW OF VOLTMETER, SHOWING TUBE COMPARTMENT

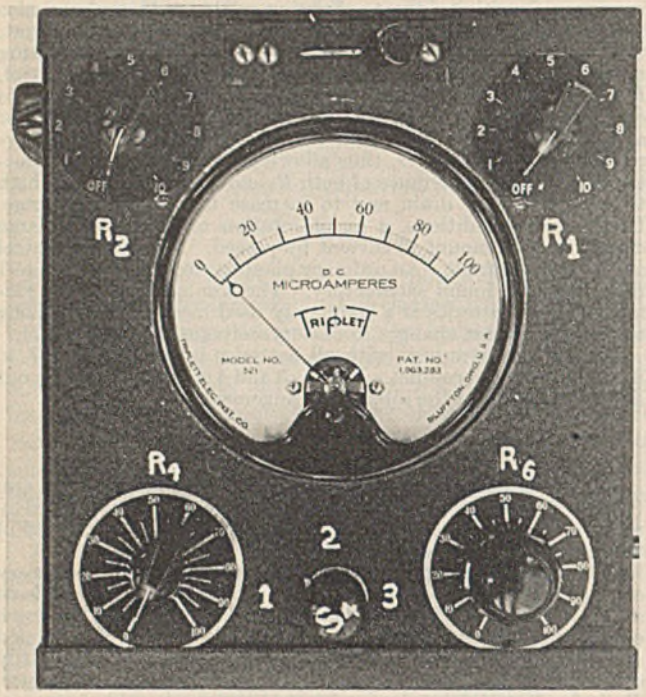

FIGURE 4. FRONT VIEW OF VOLTMETER

of any high-resistance circuit must be highly shielded and common practice is to shield the glass electrode lead. The starting position of the titration will then depend on whether this electrode is to become more positive or more negative. In order to keep the grid current low it is desirable to keep the grid potential within a certain range relative to free grid, determined by the grid current characteristics of the particular tube used. To stay within this range during a titration, the needle is reset with the bias control as it approaches the end of the meter scale. This allows the use of maximum sensitivity, if desired, over a voltage range more than equivalent to 14 pH units.

If it is desired to use the instrument as a null-point indicator, it is necessary simply to adjust the grid potential with the biasing control to the free grid potential for zero input voltage and to balance out the electrode potential with a precision potentiometer inserted in the low-resistance lead.

Operating Characteristics

The grid current of the electrometer tube must be kept low for two reasons: to ensure an accurate voltage response and to prevent appreciable polarization in the electrode system (*2*). The first of these is important only in measuring circuits employing high-resistance electrodes. For a given accuracy of voltage measurment the grid current becomes the factor which limits the resistance of the electrodes that can be used.

The prevention of polarization is important for all electrode systems. According to Müller (*20*) the limiting current depends upon the type of cell and the time of current flow. In glass electrode systems, Morton (*19*) has said that currents of 10^{-10} to 10^{-9} ampere do not cause appreciable polarization. Robertson (*27*) found in working with electrodes of 2- to 3-megohm resistance that more than 6×10^{-9} ampere was required to give measurable polarization in one minute. As Figure 5 shows, grid currents of 10^{-10} ampere are never reached in the voltage range recommended for the operation of this instrument. As an additional check with the present instrument the following experiment was performed:

With a potentiometer connected in the *EMF* circuit, the potential of a glass electrode (300-megohm resistance)–calomel cell in a buffer solution was determined. The potential was then adjusted to −0.2 volt with respect to free grid. The potential of the cell as indicated by the meter reading showed no change in 30 minutes when corrected for the instrument drift. The electrodes were then disconnected for 5 minutes. When reconnected, they gave the same potential as originally measured by null-point determination. The current flowing through the cell was about 3×10^{-11} ampere. Subsequent work with the same glass electrode has shown that no measurable polarization occurs when titrations requiring several hours for completion are carried out by the recommended procedure.

The advantages of working at the free grid potential have been thoroughly discussed by Nottingham (*21*) and Rasmussen (*26*). According to these authors, the gird current at the free grid is theoretically zero. On the positive side of the free grid, the current increases sharply, owing to an electron flow to the grid. On the negative side of the free grid, the current flow is in the opposite direction. According to Metcalf and Thompson (*17*), the principal causes of this "positive" current are: (1) ionization of residual gas, (2) positive ions emitted by the filament, (3) thermionic grid emission due to heating of the grid by the filament power, (4) photoelectric grid emission due to both visible light from the filament and x-rays produced by the anode current, and (5) insulation leakages.

By the use of a high order of insulation in the grid lead (special input jack and leads, highly insulated switch S_4, tested condenser, paraffined tube surface, and desiccant) leakage currents have been minimized. The presence of one negatively charged grid and two positively charged ones between the filament and control grid will aid in screening the latter against positive ions emitted by the filament. The extremely low filament power of the 1A7G tube (0.08 watt as compared with the 0.2 to 0.5 watt given as average for electrometer tubes, *3*) should make the thermionic and photoelectric grid emissions small for this tube. Attempts to lower the plate and anode grid voltages and thus reduce gas ionization and x-ray emission resulted in drastic loss of sensitivity. Sacrifice of sensitivity was not deemed advisable, since the grid current was already low enough for most measurements.

Figure 5 shows the grid current characteristics for three 1A7GT tubes, all of which were considered satisfactory for use in the voltmeter. Out of a total of eight 1A7GT tubes tested, six Sylvania and two Ken-Rad, only three tubes were found to be suitable for use; two of these were Sylvanias and one was a Ken-Rad. The other tubes in general had a very high $\partial Ig/\partial E$, so that high currents were found very near the free grid potential. Because of this variability in tubes it is necessary to make selection of the tubes with the instrument itself. The disadvantage of having to select the tubes is one commonly encountered in tubes not specially designed for use in electrometers. Satisfactory tubes are selected by measuring the current at the free grid potential and at suitable voltage intervals on either side of the potential by observing the meter deflection with and without a 5000-megohm resistance in the grid circuit. From the voltage sensitivity and the deflection difference the current is computed by Ohm's law.

The grid current characteristics of these tubes appear to be a function of the bridge balance. Tubes which balance at approximately equal plate and anode grid resistances have high grid currents, whereas tubes having a high plate resistance (low plate current) have a much lower grid current. This current would therefore appear to be intimately related to the amount of plate current, a not unexpected result, when we consider the sources of grid currents discussed above. Unfortunately, the conditions for the highest sensitivity correspond to those where the highest grid currents are found. The free grid current for the tubes found to be suitable was less than 3×10^{-13} ampere within the limit to which the free grid potential could be located by the procedure described. With the unsatisfactory tubes it was not possible to approach the free grid potential close enough to secure as low a current. The grid currents found make the instrument unsuitable for use with circuits having a higher resistance than 30 megohms

if an accurate voltage response of 1 millivolt is desired over the operating range indicated or with a higher resistance than 300 megohms if an accuracy of 0.1 millivolt is desired for null-point measurement. This would obviate its use in mass spectrometers or phototube outfits where the resistance is 10^9 to 10^{10} ohms. However, excellent glass electrodes ranging in resistance from 2 to 30 megohms may be purchased commercially or constructed by the experimenter (*5*, *27*).

The foregoing remarks apply to the instrument as constructed. Figure 2 shows that increasing the bridge resistance causes a proportional increase in sensitivity and decrease in range of linear response. When used as a null-point indicator or a short-range voltage indicator in connection with a potentiometer this would be no disadvantage.

The choice of meter used with the instrument would depend on the use for which it is intended. For null-point detection a 0–50 microammeter or galvanometer could be used. As a titrimeter a 0–500 microammeter is satisfactory. Both a 0–100 and a 0–500 microammeter have been used in the present instrument with excellent results. Random fluctuations in the meter readings have not been observed. When dry cells are used for the filament supply there is a drift which decreases to less than 1 microampere per minute after the instrument has been turned on for about 2 hours. The authors recommend the use of storage cells for the filament supplies where practicable, as the meter drift is reduced tenfold by this practice. In all the measurements reported in this paper storage batteries were used. Many experimenters have been successful in minimizing drift by various methods of internal compensation (*1*, *3*, *7*, *23*). However, rate of drift of the present instrument remains relatively unchanged in spite of attempts of the authors to make use of such compensation methods.

An attempt also was made to construct a simple alternating current power supply to replace the batteries and thus eliminate the drift effects. When voltage regulator tubes of the glow-discharge type were used, it was found, however, that the power delivered could not be controlled within the extremely small limits necessary for complete stability. More complicated regulating circuits, such as those of Working (*34*), would probably be satisfactory if they could deliver sufficient current.

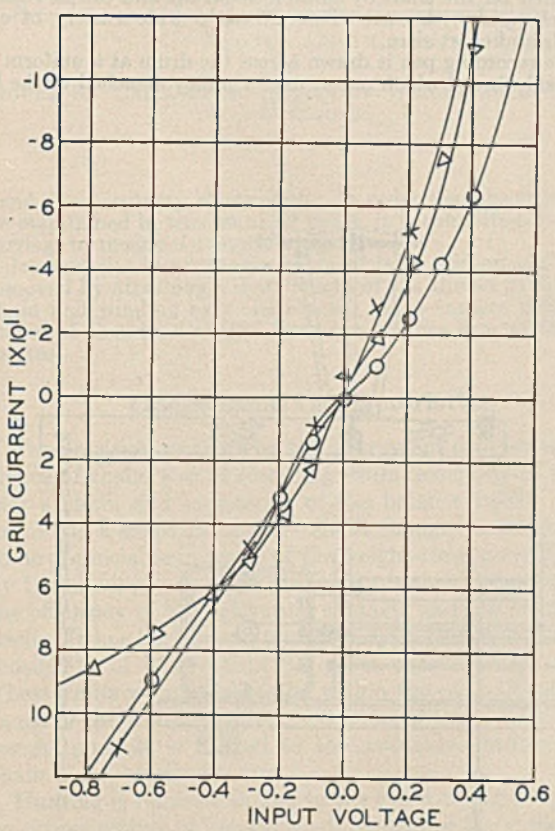

FIGURE 5. VARIATION OF GRID CURRENT WITH INPUT VOLTAGE FOR THREE SATISFACTORY TUBES

Summary

This article describes a thermionic voltmeter suitable for a wide range of laboratory measurements in both high- and low-resistance circuits.

A description of the circuit, its characteristics, and operation is given and the following uses are indicated: (1) as a direct, continuous-reading titrimeter for glass electrode systems, (2) as a direct continuous-reading titrimeter for low-resistance electrodes, and (3) as a null-point indicator in connection with a potentiometer. The instrument has proved very satisfactory in actual operation over a period of several months in each of these applications. Further applications such as the measurement of photocell currents, etc., in circuits of moderately high resistance are indicated.

The total cost of the materials used in the instrument less batteries was about $25. The particular batteries used cost $5. The low current drain ensures long life for these batteries.

Acknowledgment

The authors wish to express appreciation to E. G. Moberg of the Chemistry Division for suggestions during preparation of the manuscript, and to thank Ben Kievet and the Hygrade Sylvania Corporation for their courtesy in furnishing six life-test tubes for the grid current measurements.

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Simple Automatic and Recording Balance

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The paper describes the design, construction, and operating characteristics of a simple, rugged, automatic recording balance, which may be constructed in the average laboratory workshop. The instrument is suitable for most of the chemical engineering laboratory operations where a continuous record of sample weight is desired over long periods.

THE authors were faced with the problem of continuously weighing a sample of approximately 1 kg. during a drying test covering one day or more. The reduction in sample weight in most cases was about 0.5 kg. This indicated that a sensitivity of 0.1 gram would be sufficient and would lead to results accurate to 0.5 per cent with the sample weight varying as little as 20 grams.

Numerous balances are described in the literature (*1–5*). These are rather complex and expensive, but are reported to be extremely precise over a narrow range of weight. The range may be extended by the addition of counterweights but for the purpose in mind this would result in constant attendance. For the most part, the instruments described are not easily built in the average laboratory workshop by the student or mechanic available. It was therefore decided to design and construct an instrument which would be rugged, cover a wide range of weight (1 kg.), and still have a sensitivity of 0.1 gram.

In view of the desired sensitivity, compensating devices such as those employed by Müller and Garman (*3*) and Ewald (*2*) were dispensed with and the balance was greatly simplified. The instrument maintains the balance arm at equilibrium by reeling in or playing out sufficient chain to counteract changes in sample weight. The chain mechanism is actuated by an energized pointer which is located above the knife edges on the balance beam. The pointer makes contact with either of two adjustable plates set 0.5 cm. apart, which in turn are connected to relays operating the clockwise and counterclockwise circuits of a small motor. The recording mechanism is integral with the chain reel and a moving pen follows changes in weight continuously.

Balance and Recorder

The balance is mounted in the wall of the dryer and the compensating and recording mechanism is placed in such a position that the chain describes a smooth catenary curve between the end of the balance arm and the chain reel. Figure 1 illustrates the assembled apparatus. The sample is suspended from the end of the balance arm inside the dryer, the chain being attached to the other end of the arm outside the dryer. The balance arm is maintained at equilibrium by the addition to, or removal of, the chain from a reel operated by a small (1.5 kg.-cm., or 20 ounce-inch) reversible, 110-volt, alternating current motor of the shaded pole type.

The actuating circuit diagram is shown in Figure 2. The balance arm pointer is energized by connecting one pole of a 45-volt, dry cell battery to the top of one of the steel knife edges by a fine copper wire. A condenser is placed in parallel with the pointer to reduce sparking at the contacts. A slight change in weight causes the pointer to touch one of the contact plates which operates the relays energizing the clockwise or counterclockwise circuits of the motor. The contact plates are insulated from the supporting frame by thin sheets of Bakelite paper.

The motor is coupled to the shaft through a gear train which reduces the motor speed from 3600 to 1 r. p. m. at the shaft. Friction is reduced by mounting the shaft on ball bearings set in Bakelite supports. The recording drum and chain reel are mounted on the shaft by suitable setscrews and can be removed and changed with ease. This affords a wide variety of chain speeds and chart sizes.

The recording pen is drawn across the drum at a uniform rate by a silk thread which passes over a pulley attached to the hour

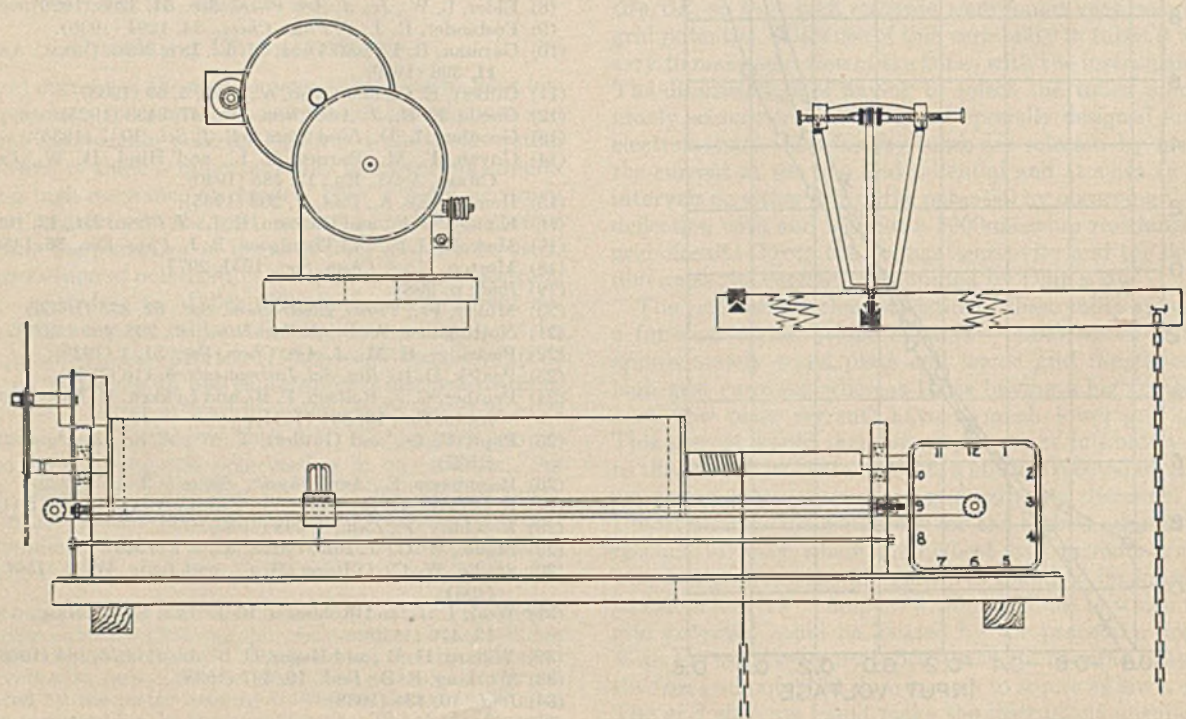

FIGURE 1. ASSEMBLED APPARATUS

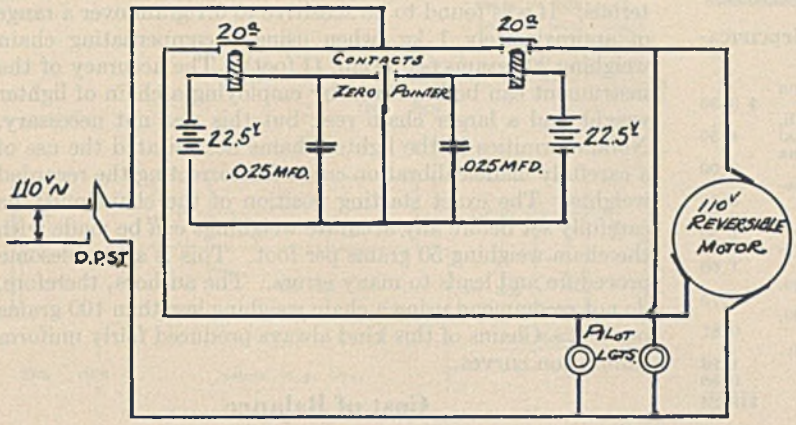

FIGURE 2. CIRCUIT DIAGRAM OF COMPENSATOR

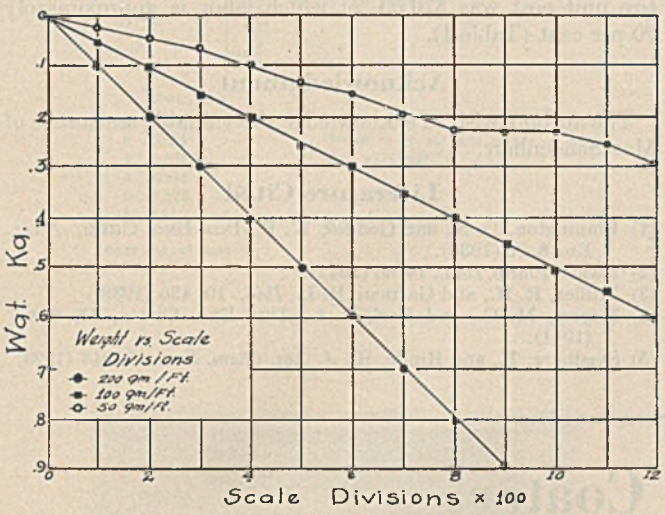

FIGURE 3. CALIBRATION CURVES OF WEIGHT AGAINST SCALE DIVISIONS

hand of an ordinary alarm clock. In order that the thread may be maintained in tension at all times, it is connected to the pen carriage by means of a small spring.

Inaccuracies in winding a chain of large size on the reel are removed by attaching a short length of silk thread to the end of chain and winding only the thread. This ensures uniform removal of the chain weight from the balance arm as the drum rotates.

Operational Characteristics

The precision attained by the instrument depends upon the choice of chain, size of recording drum, efficiency of the contact system, and sensitivity of the balance itself. A large drum and a small chain cause slight changes in weight to become appreciable in terms of the weight-time curve produced by the moving pen. The sensitivity is then dependent upon the efficiency of the activating contacts and the balance arm itself. It has been found unnecessary in this case to attain a sensitivity of better than 0.1 gram over a range of 1 kg. These limits were found to be within the over-all sensitivity range of the contact system and balance arm. The range of weight possible is limited to the available headroom for a chain of any particular size.

Hunting is reduced almost to the point of imperceptibility by proper setting of the screw adjustment of the variable contact plates. A slight amount of hunting is desirable, for it indicates that the weighing is not being made on a static beam, and the compensator is adding or removing sufficient chain to keep up with changes in sample weight.

The size of chain and drum is chosen so that each gram of sample weight corresponds in magnitude to a large scale division on the recording paper. The recording pen is moved across the paper at a rate which permits each large scale division of the paper to represent 15 minutes. This makes it easy to convert the indicated readings to terms of time and weight. By marking the position of the chain at a known weight, and setting the chain in the same position at the start of all weighings, the scale divisions of the resultant graph are made to take on absolute values.

The balance was calibrated against a standard set of analytical weights. The calibration curve of scale divisions against weight in grams is found to be a straight-line relationship over the desired range (Figure 3), provided that there are 3 or more feet of free chain hanging between the reel and balance arm. A plot of weight in grams against time indicates the desired sensitivity (Figure 4). The curves indicate that changes in weight of less than 0.1 gram are recorded by the instrument, but these were found to be unreliable, in that subsequent addition and removal of less than 0.1 gram did not result in uniform readings in all cases. With a chain

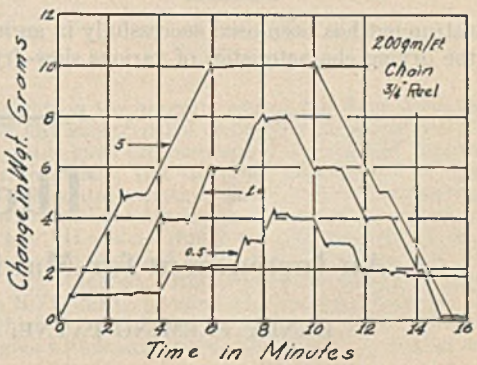

FIGURE 4. CHANGE IN WEIGHT PLOTTED AGAINST TIME REQUIRED TO REACH EQUILIBRIUM

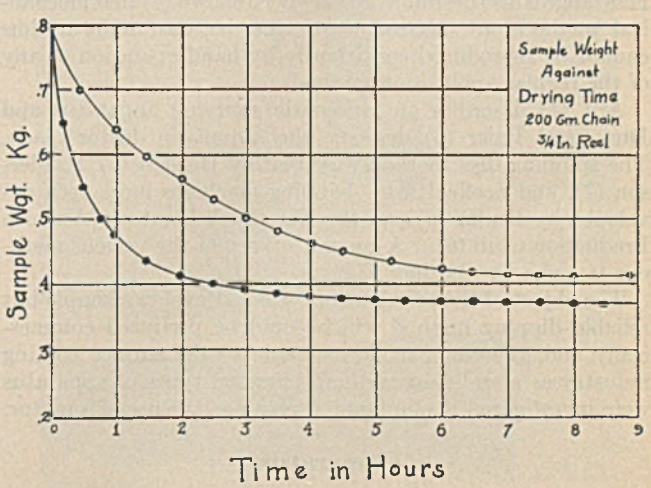

FIGURE 5. TYPICAL WEIGHT-TIME CURVES TAKEN DURING 9-HOUR DRYING TEST RUN

TABLE I. COST OF CONSTRUCTION AND MATERIAL SPECIFICATIONS

1	A. C., 20 ounce-inch, reversible motor complete with reduction gears. Utah Radio Products Corp., Chicago, Ill., or equal	$ 3.50
2	20 ampere relays, open type. Allied Radio Corp., Chicago, Ill. Cat. No. 107, p. 150, No. 75-600 type, PRA-1 SPST, or equal	4.50
1	2 × 0.25 inch recording pen. Leeds & Northrup. Micromax recording pen or equal	2.00
2	0.5 × 0.5 × 1.5 inch ball bearings. Boston Gear Works, Boston, Mass., or equal	0.75
1	0.5 × 3 inch mild steel rod, round, hot rolled	0.35
1	3 × 24 inch brass tube, seamless. Crane Co., Chicago, Ill.	1.50
2	5-inch gear blanks, aluminum (salvage from miscellaneous shop junk)	1.60
1	1 × 6 × 12 inch Bakelite board. Allied Radio Corp., Chicago, Ill., or equal	0.75
4	Assorted chains 10 feet each in weights of approximately 50, 100, 200, and 500 grams per foot	0.81
1	Burgess 45-volt, dry cell battery with plug. Allied Radio Corp., Chicago, Ill., or equal	1.98
	Miscellaneous wire, screws, etc.	0.50
		$18.24

weighing 200 grams per foot and a chain reel 1.9 cm. (0.75 inch) in diameter, the mechanism is sensitive to 0.1 gram over a range of 1100 grams.

Large ranges of weighings will cause the drum to make more than one revolution during the experiment. This is often the case when a light-weight chain is used. The record can either be cut into sections and glued together to give one long, continuous curve, or the points of the record may be transcribed on standard 20 × 27.5 cm. (8 × 11 inch) graph paper. Figure 5 illustrates some typical curves transcribed in the second manner.

Results

The instrument has been used successfully in an investigation of the drying characteristics of various slow-drying materials. It was found to be sensitive to 0.1 gram over a range of approximately 1 kg. when using a compensating chain weighing 200 grams per 30 cm. (1 foot). The accuracy of the instrument can be improved by employing a chain of lighter weight and a larger chain reel, but this was not necessary. Nonuniformities in the lighter chains necessitated the use of a carefully made calibration curve for correcting the recorded weights. The exact starting position of the chain must be carefully set before any accurate weighings can be made with the chain weighing 50 grams per foot. This is a troublesome procedure and leads to many errors. The authors, therefore, do not recommend using a chain weighing less than 100 grams per foot. Chains of this kind always produced fairly uniform calibration curves.

Cost of Balance

The cost of the balance and accessories is small, and the largest item in the construction cost is the one of labor. The materials can be purchased from standard dealers. The entire unit cost was $70.00, of which labor is approximately 70 per cent (Table I).

Acknowledgment

The authors wish to acknowledge the valuable assistance of Max Schoenherr.

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The Dip Coater

An Instrument for Making Uniform Films by the Dip Method

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IT HAS long been recognized that uniformity in thickness of a film of surface coating is one of the essential requisites for accurate evaluation of the film properties. The ability to reproduce uniform films of any thickness in the normal thickness range is also desirable. It has been shown (*6*) that mechanical methods are necessary for this, because uniform films cannot be reproduced consistently by hand operation of any of the regular application methods.

Arlt (*1*) describes an automatic spraying apparatus and Dunn and Baier (*3*) describe the automatic doctor blade. The spinning disk method was used by Haslam (*5*), Sanderson (*7*), and Scofield (*8*). Dipping machines have been developed by Bruins (*2*) and the New York Paint and Varnish Production Club (*6*). A general survey of the various methods is given by Gardner (*4*).

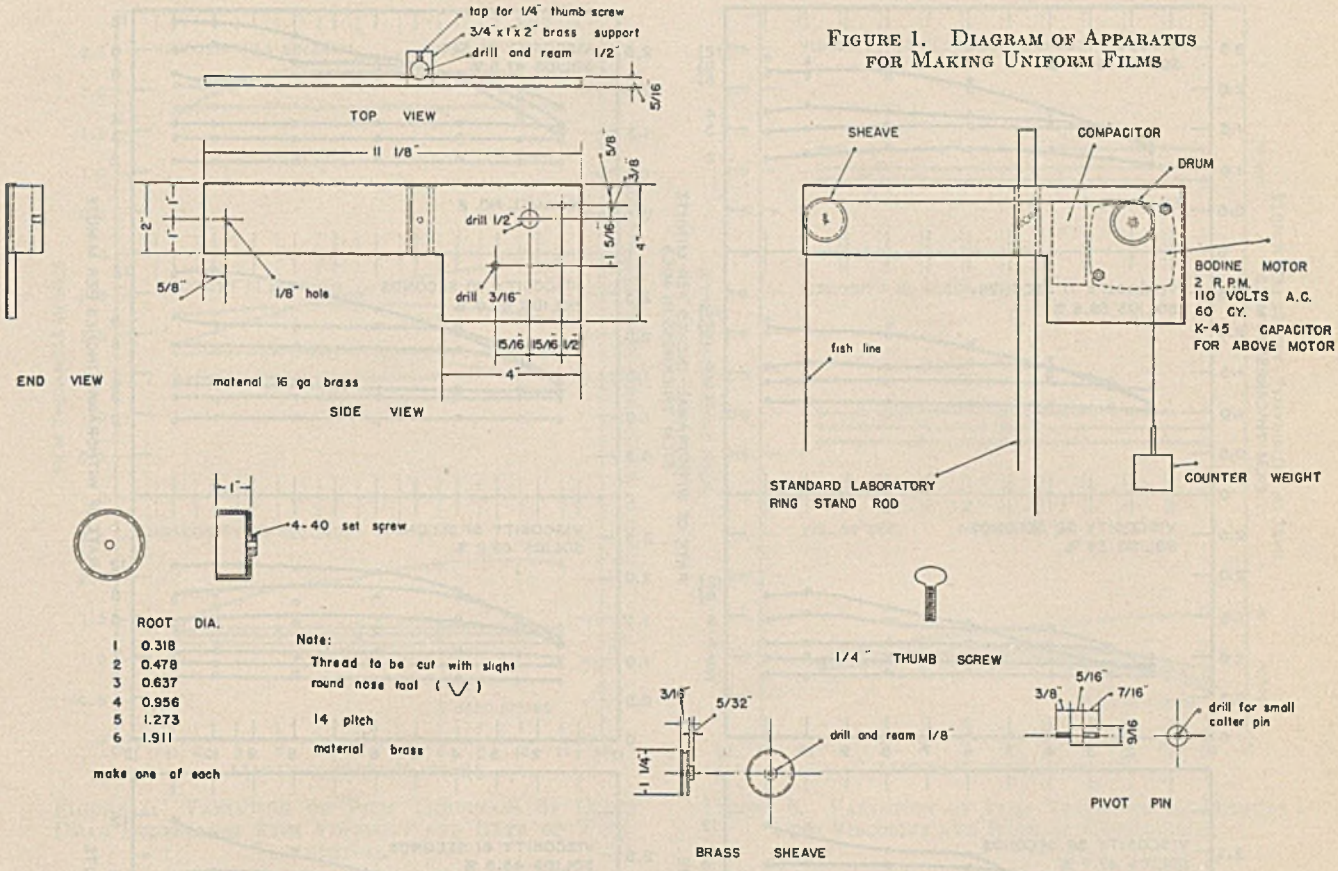
The object of the present work was to develop a simple but reliable dipping method which could be produced commercially and, therefore, made available to the surface coating industry as a standard method. Several types of apparatus were investigated to eliminate, if possible, the use of a motor.

Apparatus

The rate of fall of an object in media of different viscosities will vary with viscosity, and this may be used with suitable attachments to withdraw a panel at the different rates from a container of surface coating. Considerable difficulty was encountered in maintaining a uniform rate and reproducing results because of the relatively slow motion which was required.

An apparatus was constructed in which the container of surface coating was supported on a plunger which could be raised and lowered in a cylinder in which liquids of different viscosities were put. A definite improvement in this apparatus was made by installing a by-pass tube from the bottom to the top of the cylinder. The plunger had several holes in the disk, through which the liquid in the cylinder could flow freely when the plunger was raised, but the holes were automatically covered by a supplementary leather disk when the plunger began to lower. The rate of lowering of the plunger was then controlled by adjustment of a needle valve installed in the by-pass tube, controlling the rate of flow of the liquid from the bottom to the top of the cylinder. A low-viscosity liquid could then be used, such as kerosene, the viscosity of which is not affected seriously by normal changes in room temperature. At the low speeds required (2 to 4 inches per minute), considerable leakage developed between the leather and metal disks, so that a given speed could not be reproduced by a definite setting of the needle valve. This method would be satisfactory if metal check valves were used in the plunger disk, and the plunger and side walls of the cylinder machined with extreme accuracy.

An apparatus was then constructed similar to that shown in Figure 1, but using a clock motor. A set of Boston sprockets (Boston Gear Co.) was used to vary the speed with the standard chain drive. This type of motor has low power, but by hanging a



	ROOT DIA.
1	0.318
2	0.478
3	0.637
4	0.956
5	1.273
6	1.911

make one of each

Note:
Thread to be cut with slight round nose tool (\/)
14 pitch
material brass

FIGURE 1. DIAGRAM OF APPARATUS FOR MAKING UNIFORM FILMS

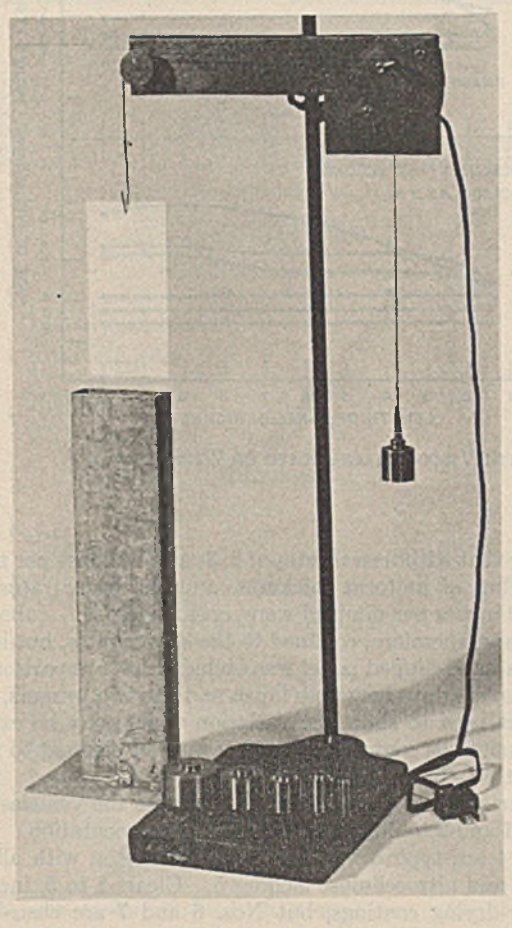


FIGURE 2

counterweight on the opposite end of the chain from the panel, practically any size of panel or number of panels could be used. This apparatus gave excellent speed control and appeared to be entirely satisfactory, but on close examination of the coated panels, a horizontal striation was found in the coating. This was caused by a slight amount of lost motion in the gear reducer of the motor. It was not due to the sprockets and chain, because when they were replaced with drum and fish line, the same striation was observed. Replacement of the clock motor with a Bodine KYC-22RC (Bodine Electric Co., Chicago, Ill.) gave results which were entirely satisfactory. A set of drums was made to give withdrawal speeds of 2, 3, 4, 6, 8, and 12 inches per

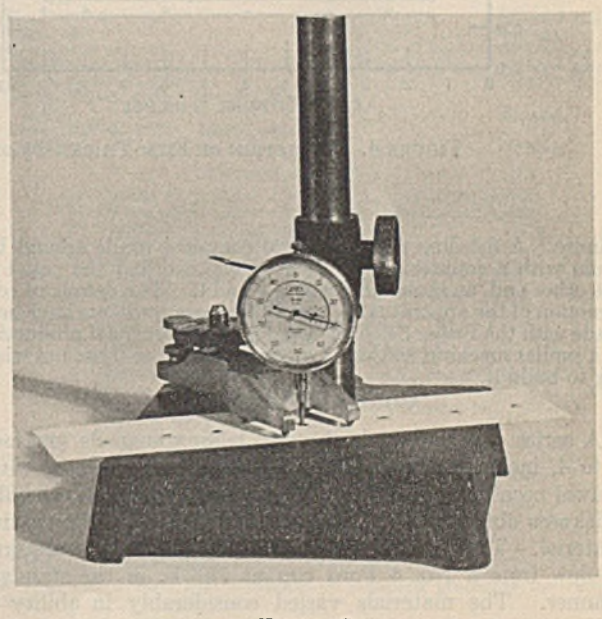


FIGURE 3

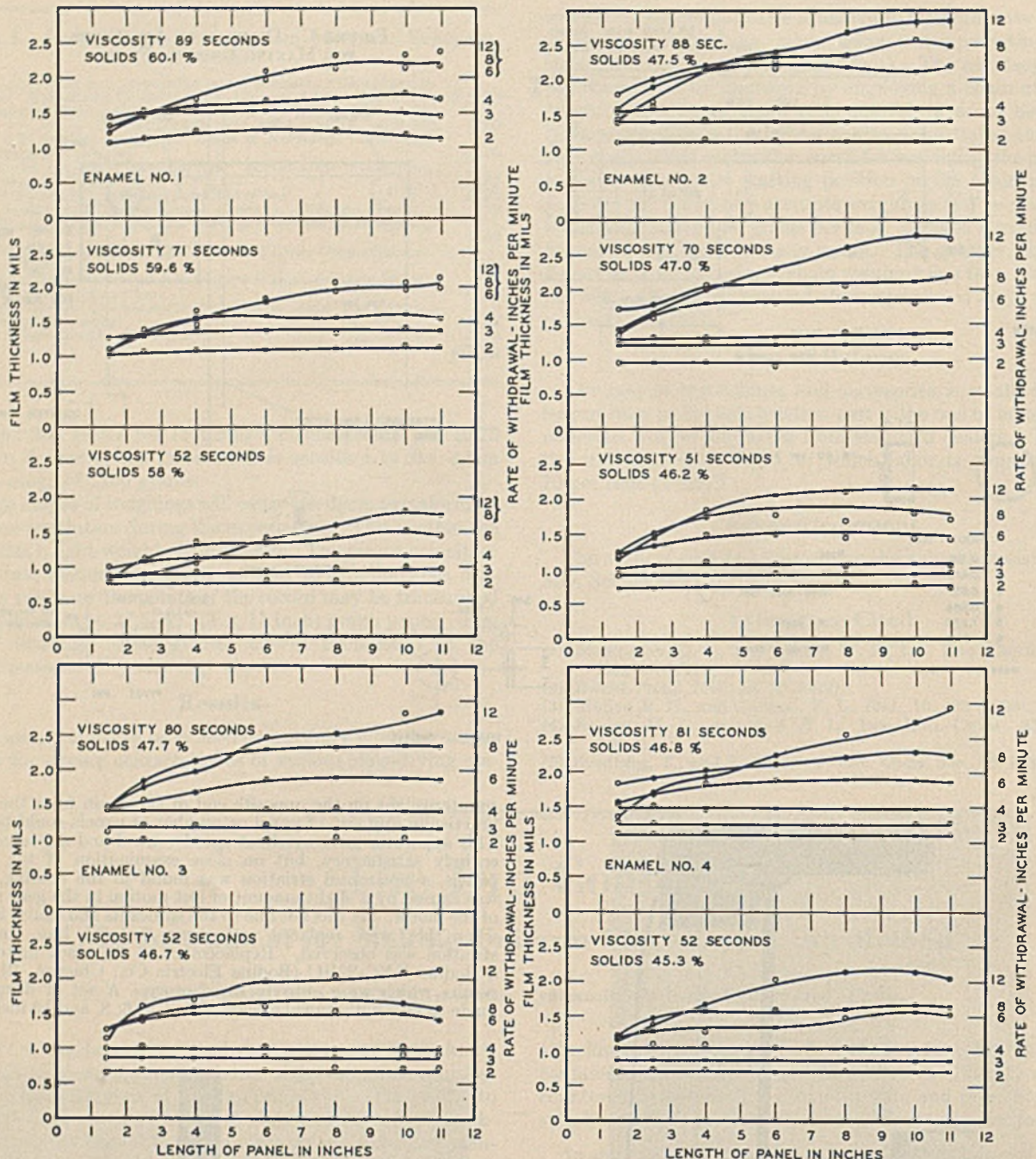


FIGURE 4. VARIATION OF FILM THICKNESS OF ENAMELS WITH VISCOSITY AND RATE OF WITHDRAWAL

minute. A fish line was given two complete winds around the drum with a counterweight hung on one end and the panel on the other end, as shown in Figures 1 and 2. The details of construction of the apparatus are shown, but arrangements have been made with the Fisher Scientific Co. for the commercial production of a similar machine, so that it will be available to those not wishing to build it.

Coating Materials

A series of four standard white baking enamels (enamels 1 to 4, inclusive) was made to determine the rate of withdrawal necessary for uniform thickness films and also the film thickness obtained with variation of viscosity of the coating material. The viscosity was measured as seconds required to flow from a No. 4 Ford cup at 78° F. in the standard manner. The materials varied considerably in ability to flow, rate of setup, and composition; but the data showed clearly that withdrawal rates of 2, 3, and 4 inches per minute gave films of uniform thickness, and the faster rates (6, 8, and 12 inches per minute) were unsatisfactory. Subsequent work was, therefore, confined to the slower rates, but in most cases a hand-dipped panel was included for comparison.

The olive drab lusterless finish and the two primers, A and B, were used to show the variation which may be expected from materials containing a higher ratio of pigment to vehicle solids than that in the enamels.

Clear coatings 1 and 2 are the same as the vehicles in enamels 1 and 2 to illustrate the effect of pigmentation. Clears 3 and 4 are typical varnishes for comparison with alkyds 1 and 2 and nitrocellulose lacquer 5. Clears 1 to 5, inclusive, are air-drying coatings, but Nos. 6 and 7 are clear-baking metal finishes, such as are used on shell cases, etc.

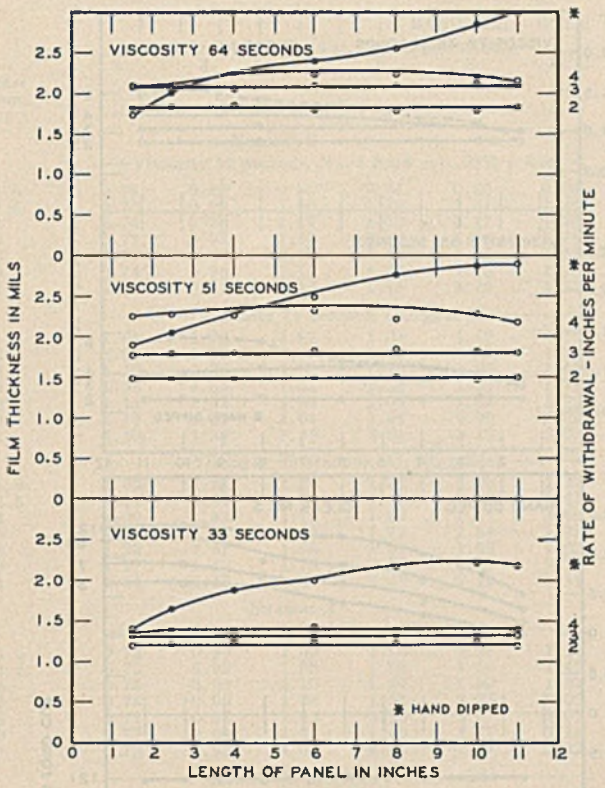


FIGURE 5. VARIATION OF FILM THICKNESS OF OLIVE DRAB LUSTERLESS WITH VISCOSITY AND RATE OF WITHDRAWAL

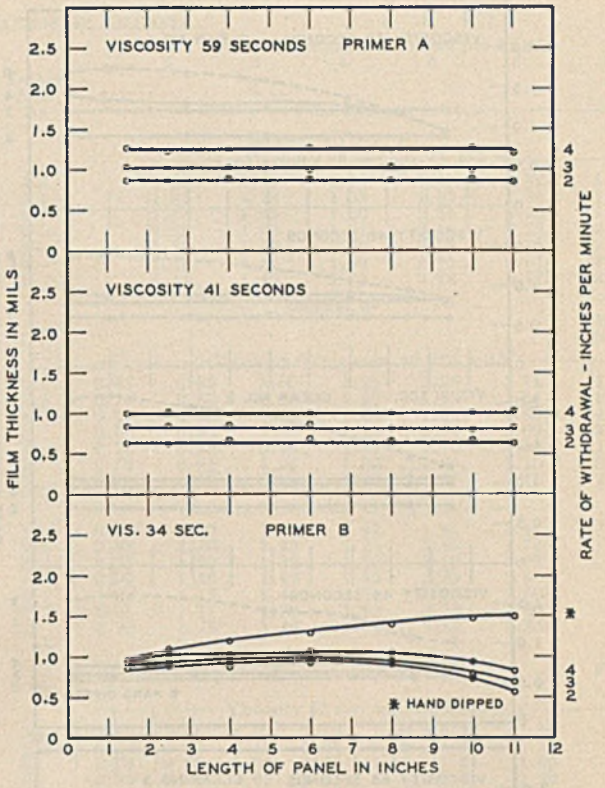


FIGURE 6. VARIATION OF FILM THICKNESS OF PRIMERS WITH VISCOSITY AND RATE OF WITHDRAWAL

TABLE I. COATING COMPOSITIONS

Pigment	White Enamels Titanium dioxide 100%
Vehicles	
Enamel 1	Rezyl 412–1[a]
Enamel 2	Rezyl 330–5
Enamel 3	Rezyl 330–5, 65%; Beetle, 227–8, 35%[b]
Enamel 4	Rezyl 330–5, 80%; Melmac 245–8, 20%[b]

Pigment–vehicle solid ratio, 1:1
Solvent
Enamel 1, mineral spirits
Enamels 2, 3, and 4, xylene

Olive Drab Lusterless

Pigment	% by Weight
Ferrite lemon	20
Medium chrome yellow	10
Zinc oxide	5
Raven black oxide	5
Asbestine 3X	30
Celite 349	25
Mica 270	5
Vehicle	
Rezyl 7818–1, 100%	

Pigment–vehicle solid ratio, 4:1
Solvent, mineral spirits

Primer A

Pigment	
Titanium dioxide	50
Zinc oxide	50
Vehicle	
Rezyl 412–1, 100%	

Pigment–vehicle solid ratio, 1.6:1
Solvent, mineral spirits

Primer B

Pigment	
Zinc yellow	7
Brown oxide	50
Zinc oxide	3
Celite 110	10
Asbestine 3X	20
Barytes	10
Vehicle	
Rezyl 7818–1, 100%	

Pigment–vehicle solid ratio, 2.7:1
Solvent, mineral spirits

[a] Trade mark, American Cyanamid Co.
[b] Trade mark, American Cyanamid & Chemical Corp.

DRYING SCHEDULES FOR COATINGS. The films were dried under following conditions before making film thickness measurements:

Enamels 1 and 2	Bake 60 minutes at 265° F.
Enamels 3 and 4	Bake 30 minutes at 265° F.
Olive drab lusterless	Air-dry 48 hours
Primer A	Bake 60 minutes at 325° F.
Primer B	Air-dry 48 hours
Clears 1 to 5	Air-dry 48 hours
Clears 6 and 7	Bake 15 minutes at 350° F.

TABLE II. CLEAR FINISHES

		Drier as % Metal to Total Solids	Solvent
1	Rezyl 412–1	0.04 Co	Mineral spirits
2	Rezyl 330–5	0.04 Co	Xylene
3	12.5-gallon varnish	0.04 Co, 0.04 Zn	Mineral spirits
4	35-gallon varnish	0.5 Pb, 0.1 Co	Mineral spirits
5	Nitrocellulose lacquer	.....	Solvent 10
6	Beetle 227–8, 60%; Beetle 592–8, 40%	.....	Xylene
7	Beetle 227–8, 70%; Rezyl 330–5, 30%	.....	Xylene

Composition of Clears

1 Drying oil modified alkyd, 32% phthalic anhydride
2 Drying oil modified alkyd, 41% phthalic anhydride
3 Maleic resin, drying oil varnish
100-lb. Teglac Z 152[a]
8.25-gal. oiticica oil (Cicoil)
4.25-gal. bodied linseed oil (KPO-Q)
4 Modified phenolic resin, drying oil varnish
100-lb. Phenac 604 M[b]
7-gal. treated fish oil (Celesterol)
18-gal. dehydrated castor oil (Isoline Z3)
10-gal. bodied linseed oil (KPO-A)
5 Nitrocellulose lacquer (parts by weight on solid basis)

0.5-sec. nitrocellulose	100	Dibutylphthalate	25
Rezyl 99–4	200	Blown castor oil	25

Solvent 10 (parts by volume)

Toluene	4	Butyl acetate	2
Ethyl acetate	3	Butanol	1

6 Nonoxidizing alkyd plasticized urea-formaldehyde baking finish
7 Oxidizing alkyd plasticized urea-formaldehyde baking finish

[a] Trade mark, American Cyanamid Co.
[b] Trade mark, American Cyanamid & Chemical Corp.

1, 2, 3, and 4 were heat-processed by standard procedure for varnish making.

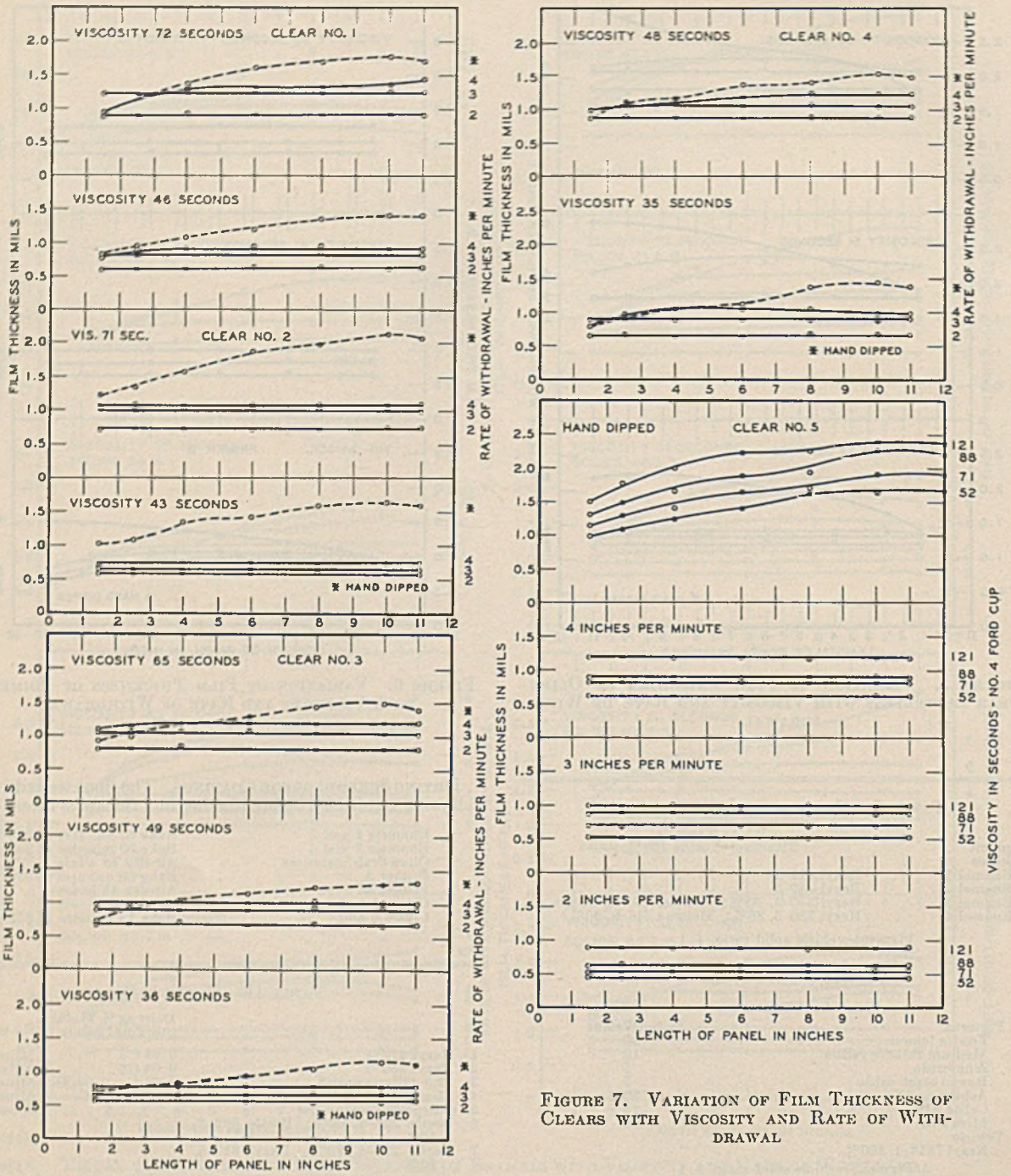


FIGURE 7. VARIATION OF FILM THICKNESS OF CLEARS WITH VISCOSITY AND RATE OF WITHDRAWAL

The air dry condition was maintained constant at 78° F., and 50 per cent relative humidity.

Procedure

All panels were dipped under conditions of constant temperature and humidity, 78° F., 50 per cent relative humidity. Cold-rolled steel panels (auto body stock) were cleaned thoroughly with toluene, and dried in a stream of compressed air free from oil or moisture. The cleaned panel was hung on a small wire hook attached to the fish line and lowered by hand into the container of surface coating to a depth of approximately 0.25 inch below the surface of the coating. The panel was then withdrawn by the dip coater at a speed dependent on the size of drum used. The drums of various size are interchangeable on the motor shaft by simple adjustment of the setscrew. The coated panels were maintained in a vertical position and dried as shown in the above schedule.

The film thickness was measured by the dial gage method (Figure 3). The coated panel was held firmly to the bed plate at four points by a special clamp shown in the figure. The total thickness (film and panel) was measured, the film removed by solvent, and the thickness of the panel determined without changing the position of the panel under the gage. The film thickness is the difference between the first and second measurements. It is extremely important that the panel be held rigidly; the clamp was designed especially for this work. It is also desirable that any slight distortion in the metal panel be eliminated. This may be done by having a threaded steel pin with a slightly rounded end protrude through the bed plate about 0.001 inch directly under the dial gage spindle. An Ames upright gage No. 13 was used in this work. The film thickness may be measured by any other method of sufficient accuracy, such as the microscope method described by Stoppel (*9*).

The film thickness was measured at seven points along the 12-inch panels with the results shown in Tables III to VII.

Table III. Film Thickness of Enamels

Inches from top	Rate of Withdrawal, Inches per Minute: 2	3	4	6	8	12
	Film thickness in mils					
Enamel 1						
	Viscosity 52 seconds, No. 4 Ford cup, 58% solids					
1.5	0.78	0.86	0.96	0.81	0.86	0.88
2.5	0.75	0.90	1.10	1.10	1.01	1.20
4	0.74	0.90	1.14	1.33	1.17	1.23
6	0.77	0.93	1.12	1.33	1.38	1.41
8	0.77	0.96	1.15	1.42	1.55	1.57
10	0.74	0.96	1.12	1.50	1.76	1.73
11	0.75	0.95	1.13	1.43	1.73	1.70
	Viscosity 71 seconds, 59.6% solids					
1.5	1.01	1.26	1.07	1.05	1.10	1.16
2.5	1.05	1.32	1.37	1.32	1.30	1.38
4	1.10	1.36	1.53	1.42	1.65	1.60
6	1.08	1.35	1.57	1.78	1.80	1.77
8	1.12	1.31	1.54	2.03	1.99	1.90
10	1.13	1.37	1.61	1.94	2.06	2.02
11	1.10	1.37	1.55	2.01	2.10	2.02
	Viscosity 89 seconds, 60.1% solids					
1.5	1.05	1.41	1.24	1.21	1.29	1.27
2.5	1.11	1.54	1.50	1.47	1.43	1.44
4	1.20	1.53	1.70	1.63	1.61	1.65
6	1.20	1.53	1.65	1.79	1.84	1.98
8	1.22	1.52	1.68	1.99	1.98	2.06
10	1.17	1.49	1.76	2.29	2.10	2.14
11	1.14	1.45	1.67	2.35	2.19	2.14
Enamel 2						
	Viscosity 51 seconds, 46.2% solids					
1.5	0.73	0.90	1.00	1.20	1.23	1.17
2.5	0.72	0.93	1.08	1.32	1.50	1.42
4	0.75	0.90	1.10	1.49	1.80	1.83
6	0.74	0.95	1.08	1.50	1.77	2.06
8	0.77	0.93	1.02	1.43	1.67	2.09
10	0.77	0.94	1.06	1.40	1.77	2.13
11	0.72	0.95	1.04	1.41	1.67	2.12
	Viscosity 70 seconds, 47.0% solids					
1.5	0.95	1.28	1.35	1.35	1.73	1.42
2.5	0.95	1.18	1.32	1.61	1.77	1.65
4	0.94	1.23	1.31	1.80	2.07	2.00
6	0.90	1.20	1.30	1.80	2.09	2.28
8	0.96	1.20	1.38	1.87	2.05	2.56
10	0.95	1.15	1.36	1.80	2.10	2.76
11	0.92	1.18	1.37	1.86	2.20	2.70

Inches from top	Rate of Withdrawal, Inches per Minute: 2	3	4	6	8	12
	Film thickness in mils					
Enamel 2 (Con't.)						
	Viscosity 88 seconds, 47.5% solids					
1.5	1.08	1.40	1.54	1.60	1.82	1.48
2.5	1.08	1.38	1.63	1.92	1.85	1.70
4	1.15	1.42	1.60	2.15	2.20	2.15
6	1.13	1.39	1.61	2.16	2.30	2.38
8	1.15	1.40	1.60	2.18	2.35	2.67
10	1.12	1.42	1.59	2.10	2.57	2.82
11	1.12	1.42	1.58	2.18	2.47	2.82
Enamel 3[a]						Hand Dipped
	Viscosity 52 seconds, 46.7% solids					
1.5	0.65	0.85	1.00	1.25	1.25	1.14	1.18
2.5	0.70	0.85	1.03	1.42	1.45	1.64	1.70
4	0.67	0.88	1.00	1.50	1.59	1.68	1.72
6	0.68	0.88	1.03	1.48	1.60	2.00	1.77
8	0.72	0.88	0.96	1.53	1.70	2.03	1.90
10	0.72	0.91	1.01	1.50	1.65	2.10	2.04
11	0.72	0.91	0.96	1.40	1.57	2.15	2.25
	Viscosity 80 seconds, 47.7% solids					
1.5	0.95	1.10	1.40	1.47	1.47	1.47	..
2.5	0.98	1.20	1.40	1.55	1.85	1.78	..
4	0.98	1.20	1.39	1.70	2.30	1.98	..
6	0.99	1.20	1.42	1.85	2.30	2.46	..
8	1.02	1.16	1.45	1.88	2.30	2.50	..
10	1.01	1.17	1.38	1.88	2.35	2.80	..
11	0.98	1.18	1.40	1.86	2.35	2.85	..
Enamel 4							Flowed On
	Viscosity 52 seconds, 45.3% solids						
1.5	0.70	0.88	1.03	1.19	1.20	1.15	1.10	1.41
2.5	0.73	0.87	1.10	1.27	1.48	1.37	1.40	1.55
4	0.74	0.88	1.08	1.25	1.56	1.62	1.62	1.71
6	0.70	0.88	1.05	1.32	1.58	2.00	1.80	2.01
8	0.70	0.87	1.04	1.47	1.60	2.10	2.01	2.09
10	0.73	0.90	1.04	1.58	1.68	2.13	2.15	2.17
11	0.73	0.88	1.03	1.52	1.65	2.04	2.12	2.22
	Viscosity 81 seconds, 46.8% solids						
1.5	1.10	1.27	1.53	1.55	1.83	1.35	..	..
2.5	1.09	1.24	1.50	1.68	1.90	1.72	..	..
4	1.10	1.24	1.45	1.83	2.02	1.92	..	..
6	1.10	1.22	1.45	1.85	2.11	2.22	..	..
8	1.08	1.25	1.44	1.85	2.17	2.51	..	..
10	1.10	1.27	1.44	1.87	2.29	2.70	..	..
11	1.09	1.28	1.44	1.87	2.73	2.92	..	..

[a] Enamel 3. Material flowed on had practically the same thickness as hand dipped and 12 inches per minute withdrawal.

Table IV. Film Thickness

Inches from top	Rate of Withdrawal, 2 Inches per Minute: Primer enamel, Mils	Enamel, Mils	Enamel bare steel[a], Mils	Diff., Mil	%	Rate of Withdrawal, 3 Inches per Minute: Primer enamel, Mils	Enamel, Mils	Enamel bare steel[a], Mils	Diff., Mil	%	Rate of Withdrawal, 4 Inches per Minute: Primer enamel, Mils	Enamel, Mils	Enamel bare steel[a], Mils	Diff., Mil	%
Enamel 3 over Primer A															
Viscosity 52 seconds, primer 1.00 mil															
1.5	2.10	1.10	0.65	0.45	69.2	2.32	1.32	0.85	0.47	55.3	2.51	1.51	1.00	0.51	51.0
2.5	2.10	1.10	0.70	0.40	57.3	2.34	1.34	0.85	0.49	57.6	2.53	1.53	1.03	0.56	54.2
4	2.08	1.08	0.67	0.41	61.2	2.35	1.35	0.88	0.47	53.4	2.58	1.58	1.00	0.58	58.0
6	2.15	1.15	0.68	0.47	69.1	2.38	1.38	0.88	0.50	56.9	2.58	1.58	1.03	0.53	51.4
8	2.02	1.02	0.72	0.30	41.7	2.37	1.37	0.88	0.49	55.6	2.54	1.54	0.96	0.58	60.4
10	2.15	1.15	0.72	0.43	59.7	2.34	1.34	0.91	0.43	47.4	2.52	1.52	1.01	0.53	52.4
11	2.07	1.07	0.72	0.45	62.5	2.37	1.37	0.91	0.46	50.8	2.46	1.46	0.96	0.50	52.0
Viscosity 80 seconds, primer 1.00 mil															
1.5	2.45	1.45	0.95	0.50	52.6	2.74	1.74	1.10	0.64	58.0	3.06	2.06	1.40	0.66	47.0
2.5	2.52	1.52	0.98	0.54	55.0	2.76	1.76	1.20	0.54	45.0	3.12	2.12	1.40	0.72	51.4
4	2.46	1.46	0.98	0.48	48.0	2.79	1.79	1.20	0.59	49.1	3.05	2.05	1.39	0.66	47.5
6	2.47	1.47	0.99	0.48	48.5	2.75	1.75	1.20	0.55	45.7	3.08	2.08	1.42	0.66	46.5
8	2.43	1.43	1.02	0.41	40.1	2.76	1.76	1.16	0.60	51.6	3.10	2.10	1.45	0.65	44.9
10	2.43	1.43	1.01	0.42	41.5	2.77	1.77	1.17	0.60	51.2	3.07	2.07	1.38	0.69	50.0
11	2.45	1.45	0.98	0.47	48.0	2.80	1.80	1.18	0.62	52.5	3.11	2.11	1.40	0.71	50.7
Enamel 4 over Primer A															
Viscosity 52 seconds, primer 1.00 mil															
1.5	1.84	0.84	0.70[b]	0.14	20.0	2.02	1.02	0.88[b]	0.14	15.9	2.32	1.32	1.03[b]	0.29	28.0
2.5	1.84	0.84	0.73	0.11	15.1	2.06	1.06	0.87	0.19	21.9	2.38	1.38	1.10	0.28	25.5
4	1.85	0.85	0.74	0.11	14.9	2.00	1.00	0.88	0.12	13.6	2.35	1.35	1.08	0.27	25.0
6	1.83	0.83	0.70	0.13	18.6	2.02	1.02	0.88	0.14	15.9	2.36	1.36	1.05	0.31	29.4
8	1.87	0.87	0.70	0.17	24.3	2.03	1.03	0.87	0.16	18.4	2.32	1.32	1.04	0.28	26.9
10	1.86	0.86	0.73	0.13	17.8	2.02	1.02	0.90	0.12	13.4	2.36	1.36	1.04	0.32	30.6
11	1.85	0.85	0.73	0.12	16.5	2.05	1.05	0.88	0.17	19.3	2.32	1.32	1.03	0.29	28.2
Viscosity 81 seconds, primer 1.00 mil															
1.5	2.18	1.18	1.10	0.08	7.3	2.73	1.73	1.27	0.46	36.1	2.96	1.96	1.53	0.43	28.1
2.5	2.18	1.18	1.09	0.09	8.2	2.78	1.78	1.24	0.56	45.0	2.96	1.96	1.50	0.46	30.6
4	2.22	1.22	1.10	0.12	10.9	2.76	1.76	1.24	0.52	42.0	2.97	1.97	1.45	0.52	35.8
6	2.20	1.20	1.10	0.10	10.0	2.79	1.79	1.22	0.57	46.7	2.98	1.98	1.45	0.53	36.4
8	2.20	1.20	1.08	0.12	11.1	2.75	1.75	1.25	0.50	40.0	3.02	2.02	1.44	0.57	39.5
10	2.20	1.20	1.10	0.10	10.0	2.76	1.76	1.27	0.49	38.6	2.98	1.98	1.44	0.54	37.5
11	2.20	1.20	1.09	0.11	10.1	2.74	1.74	1.28	0.46	36.0	2.96	1.96	1.44	0.52	36.2

[a] Values taken from enamel 3, Table III. [b] Values taken from enamel 4, Table III.

TABLE V. FILM THICKNESS OF OLIVE DRAB LUSTERLESS

Inches from top	Film Thickness: Rate of withdrawal, inches per minute 2	3	4	Hand dipped
	Mils	*Mils*	*Mils*	*Mils*
	Viscosity 33 seconds			
1.5	1.20	1.31	1.34	1.42
2.5	1.21	1.35	1.36	1.64
4	1.24	1.33	1.38	1.88
6	1.25	1.34	1.44	2.00
8	1.24	1.33	1.41	2.17
10	1.25	1.34	1.38	2.21
11	1.20	1.34	1.38	2.17
	Viscosity 51 seconds			
1.5	1.48	1.79	2.25	1.89
2.5	1.50	1.82	2.28	2.05
4	1.50	1.80	2.27	2.21
6	1.52	1.84	2.33	2.50
8	1.52	1.86	2.22	2.77
10	1.48	1.82	2.28	2.89
11	1.49	1.80	2.16	2.90
	Viscosity 64 seconds			
1.5	1.83	2.08	2.07	1.72
2.5	1.85	2.08	2.08	2.04
4	1.87	2.04	2.24	2.28
6	1.82	2.10	2.25	2.42
8	1.81	2.10	2.26	2.82
10	1.80	2.13	2.20	2.86
11	1.82	2.10	2.12	3.00

TABLE VI. FILM THICKNESS OF PRIMERS

Inches from top	Rate of withdrawal, inches per minute 2	3	4	Hand dipped
	Mil	*Mils*	*Mils*	*Mils*
	PRIMER A			
	Viscosity 41 seconds, 58.3% solids			
1.5	0.63	0.83	0.98	
2.5	0.63	0.85	1.02	
4	0.67	0.86	1.00	
6	0.68	0.86	0.99	
8	0.66	0.80	1.00	
10	0.67	0.79	1.01	
11	0.63	0.83	1.03	
	Viscosity 59 seconds			
1.5	0.85	1.02	1.26	
2.5	0.88	1.02	1.23	
4	0.90	1.02	1.25	
6	0.88	1.00	1.26	
8	0.86	1.05	1.25	
10	0.89	1.00	1.27	
11	0.86	1.02	1.22	
	PRIMER B			
	Viscosity 34 seconds			
1.5	0.84	0.89	0.95	0.87
2.5	0.90	0.95	1.05	1.08
4	0.86	0.98	1.03	1.18
6	0.92	0.98	1.07	1.29
8	0.90	0.93	1.05	1.38
10	0.72	0.78	0.94	1.47
11	0.58	0.69	0.84	1.50

Discussion of Data

The results with enamels 1 to 4 show clearly that withdrawal speeds greater than 4 inches per minute do not produce uniform films. Enamel 1, with mineral spirits solvent and slower setting than the others, is not entirely uniform at the 4-minute rate nor at the high viscosity. Viscosity is much more important than the per cent of solids in determining the film thickness.

Comparison of enamel 1 (71 seconds) and clear 1 (72 seconds), having the same vehicle, shows that approximately equal film thickness is produced under the same conditions of viscosity and rate of withdrawal. The greater irregularity of the clear films at 4-inch withdrawal rate is evidence of the better flowing quality of the clear unpigmented material. Primer A, having the same vehicle but a greater pigment ratio, has less flowing quality than either enamel 1 or clear 1; therefore uniform films are obtained at the 4-inch rate. Highly pigmented coatings tend to become plastic or thixotropic with very little viscous flow. It then becomes increasingly difficult to produce uniform films by the dip method. The decreased flow may be overcome partially by lowering the consistency, but this has definite limitations. The olive drab lusterless, with considerably greater pigment ratio and different type of pigment, requires lower viscoisty for uniform films of normal thickness. The pigment ratio in primer B is less than the olive drab, but this pigment has a definite tendency to flocculate, resulting in thixotropic consistency and

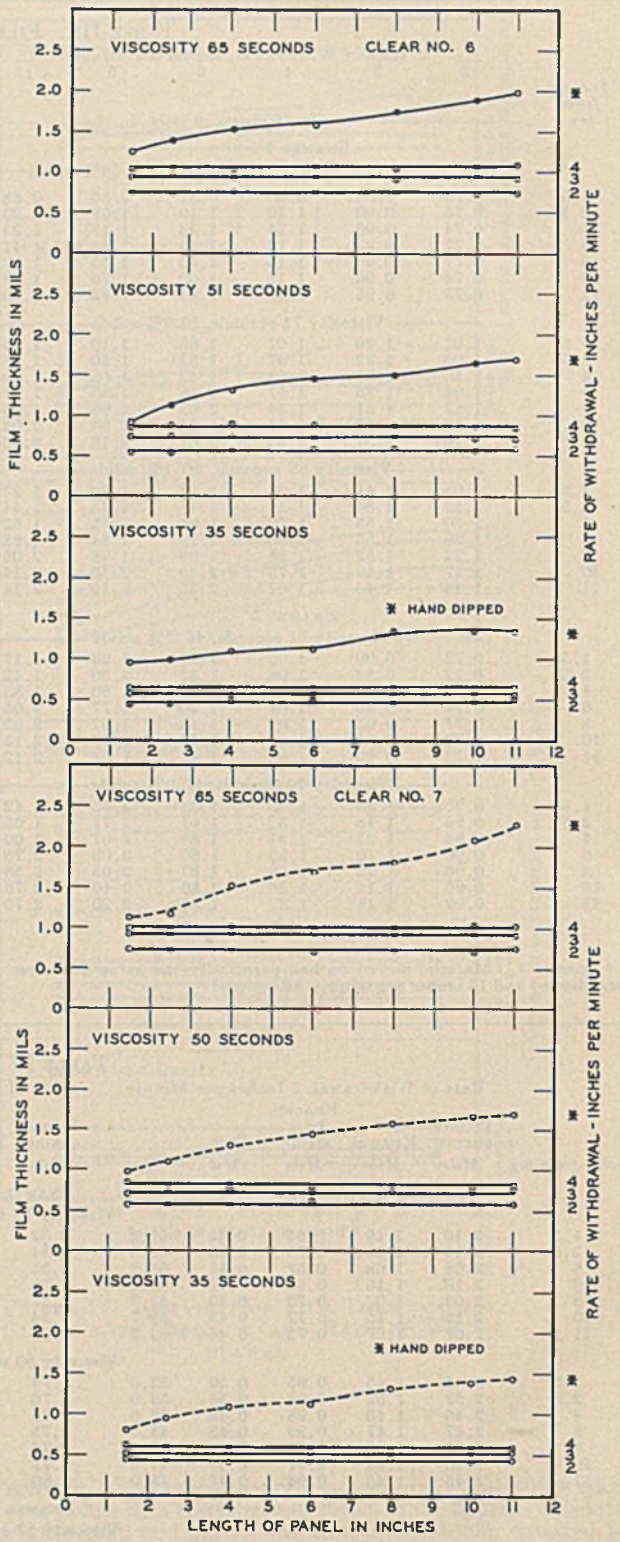


FIGURE 8. VARIATION OF FILM THICKNESS OF CLEARS WITH VISCOSITY AND RATE OF WITHDRAWAL

TABLE VII. FILM THICKNESS OF CLEARS

Inches from top	Rate of Withdrawal, Inches per Minute 2	3	4	Hand dipped
	Mil	*Mils*	*Mils*	*Mils*
CLEAR 1				
	Viscosity 46 seconds			
1.5	0.60	0.82	0.79	0.79
2.5	0.62	0.82	0.90	0.94
4	0.64	0.85	0.92	1.04
6	0.65	0.86	0.95	1.21
8	0.64	0.83	0.96	1.36
10	0.61	0.82	0.90	1.40
11	0.63	0.83	0.91	1.40
	Viscosity 72 seconds			
1.5	0.89	1.23	0.92	0.99
2.5	0.89	1.20	1.15	1.12
4	0.94	1.22	1.30	1.30
6	0.90	1.25	1.27	1.60
8	0.86	1.23	1.32	1.68
10	0.89	1.25	1.34	1.78
11	0.88	1.23	1.42	1.70
CLEAR 2				
	Viscosity 43 seconds			
1.5	0.60	0.67	0.73	1.02
2.5	0.59	0.66	0.72	1.07
4	0.63	0.64	0.78	1.35
6	0.60	0.65	0.76	1.43
8	0.61	0.66	0.75	1.61
10	0.60	0.61	0.74	1.64
11	0.60	0.62	0.75	1.60
	Viscosity 71 seconds			
1.5	0.74	1.05	1.09	1.25
2.5	0.75	1.02	1.10	1.36
4	0.74	1.04	1.09	1.61
6	0.73	1.04	1.10	1.90
8	0.74	1.05	1.09	1.99
10	0.76	1.02	1.08	2.15
11	0.76	1.00	1.10	2.08

Inches from top	Rate of Withdrawal, Inches per Minute 2	3	4	Hand dipped
	Mil	*Mils*	*Mils*	*Mils*
CLEAR 3				
	Viscosity 36 seconds			
1.5	0.59	0.65	0.75	0.72
2.5	0.60	0.65	0.78	0.80
4	0.60	0.67	0.80	0.85
6	0.57	0.68	0.80	0.96
8	0.55	0.70	0.79	1.06
10	0.55	0.67	0.76	1.15
11	0.57	0.66	0.77	1.10
	Viscosity 49 seconds			
1.5	0.67	0.91	0.99	0.73
2.5	0.69	0.91	0.99	0.89
4	0.72	0.90	1.00	1.04
6	0.68	0.90	1.02	1.15
8	0.70	0.95	1.05	1.24
10	0.68	0.95	1.03	1.30
11	0.70	0.93	1.02	1.34
	Viscosity 65 seconds			
1.5	0.81	1.03	1.10	0.93
2.5	0.83	1.07	1.15	0.98
4	0.85	1.07	1.18	1.10
6	0.82	1.07	1.20	1.30
8	0.81	1.06	1.20	1.45
10	0.82	1.05	1.23	1.49
11	0.80	1.03	1.20	1.40
CLEAR 4				
	Viscosity 35 seconds			
1.5	0.64	0.90	0.80	0.80
2.5	0.67	0.90	0.97	0.92
4	0.66	0.89	1.06	1.08
6	0.68	0.90	1.04	1.15
8	0.70	0.88	1.03	1.38
10	0.70	0.87	0.98	1.44
11	0.69	0.90	0.99	1.40
	Viscosity 48 seconds			
1.5	0.86	0.99	0.98	0.87
2.5	0.90	1.09	1.05	1.11
4	0.89	1.09	1.10	1.18
6	0.86	1.05	1.20	1.37
8	0.90	1.07	1.25	1.41
10	0.89	1.06	1.25	1.54
11	0.88	1.06	1.25	1.48

Inches from top	Rate of Withdrawal, Inches per Minute 2	3	4	Hand dipped
	Mil	*Mils*	*Mils*	*Mils*
CLEAR 5				
	Viscosity 52 seconds			
1.5	0.46	0.52	0.63	0.99
2.5	0.45	0.53	0.62	1.10
4	0.48	0.52	0.63	1.24
6	0.46	0.53	0.61	1.41
8	0.44	0.52	0.61	1.63
10	0.44	0.53	0.64	1.64
11	0.44	0.54	0.61	1.65
	Viscosity 71 seconds			
1.5	0.55	0.70	0.83	1.15
2.5	0.55	0.70	0.83	1.29
4	0.54	0.70	0.83	1.41
6	0.56	0.69	0.84	1.65
8	0.56	0.68	0.83	1.70
10	0.55	0.70	0.82	1.90
11	0.55	0.70	0.82	1.90
	Viscosity 88 seconds			
1.5	0.64	0.87	0.90	1.33
2.5	0.65	0.90	0.90	1.50
4	0.63	0.89	0.91	1.66
6	0.65	0.90	0.90	1.87
8	0.65	0.87	0.91	1.93
10	0.65	0.86	0.92	2.27
11	0.65	0.88	0.90	2.20
	Viscosity 121 seconds			
1.5	0.90	1.00	1.22	1.50
2.5	0.90	1.00	1.20	1.77
4	0.89	1.02	1.20	2.00
6	0.88	1.00	1.19	2.23
8	0.87	1.01	1.18	2.50
10	0.89	0.98	1.19	2.65
11	0.90	0.98	1.17	2.60

Inches from top	Rate of Withdrawal, Inches per Minute 2	3	4	Hand dipped
	Mil	*Mil*	*Mils*	*Mils*
CLEAR 6				
	Viscosity 35 seconds			
1.5	0.55	0.59	0.66	0.95
2.5	0.46	0.58	0.64	0.98
4	0.50	0.57	0.66	1.10
6	0.50	0.59	0.64	1.12
8	0.50	0.58	0.66	1.35
10	0.50	0.60	0.65	1.35
11	0.50	0.57	0.65	1.34
	Viscosity 51 Seconds			
1.5	0.55	0.74	0.84	0.93
2.5	0.55	0.75	0.89	1.12
4	0.58	0.75	0.90	1.31
6	0.62	0.73	0.88	1.46
8	0.60	0.75	0.88	1.49
10	0.56	0.72	0.86	1.66
11	0.60	0.70	0.85	1.68
	Viscosity 65 seconds			
1.5	0.74	0.95	1.05	1.25
2.5	0.75	0.95	1.06	1.40
4	0.78	0.92	1.04	1.53
6	0.76	0.93	1.07	1.57
8	0.76	0.92	1.05	1.75
10	0.74	0.95	1.07	1.88
11	0.75	0.92	1.10	1.98
CLEAR 7				
	Viscosity 35 seconds			
1.5	0.46	0.53	0.63	0.81
2.5	0.45	0.55	0.59	0.96
4	0.42	0.56	0.60	1.10
6	0.42	0.54	0.59	1.13
8	0.48	0.55	0.58	1.33
10	0.41	0.53	0.59	1.37
11	0.43	0.53	0.59	1.44
	Viscosity 50 seconds			
1.5	0.60	0.73	0.86	1.00
2.5	0.59	0.75	0.82	1.11
4	0.59	0.77	0.84	1.31
6	0.63	0.74	0.81	1.47
8	0.60	0.75	0.79	1.59
10	0.60	0.75	0.82	1.67
11	0.60	0.78	0.82	1.70
	Viscosity 65 seconds			
1.5	0.73	0.93	1.00	1.12
2.5	0.72	0.92	1.00	1.17
4	0.73	0.92	1.02	1.52
6	0.71	0.92	1.01	1.69
8	0.72	0.92	0.98	1.80
10	0.71	0.92	1.02	2.08
11	0.75	0.91	1.03	2.28

irregularity in film thickness. This material is not entirely satisfactory for dip coating and shows that the type of pigment and degree of dispersion must be considered to obtain satisfactory results.

The extreme variation in film uniformity on the panels which were hand-dipped makes this method very unsatisfactory for making test panels. The effect of slow setting produced by mineral spirits may be seen again in a comparison of clears 1 and 2. It is particularly evident in the hand-dipped panels. Clears 3 and 4 are both thinned with mineral spirits, but No. 4 is slower setting than No. 3 because of a greater ratio of oil to resin. The greater irregularity of No. 4 at comparable viscosities and the 4-inch rate of withdrawal is at once apparent. The extreme opposite to this is the fast-setting clear No. 5, with which very uniform films were obtained at the 4-inch rate even at high viscosity. However, uniform films were not obtained with this material by the hand-dipped method.

Specification ASX-736 for shell case finishes requires a film thickness of 0.6 ± 0.1 mil. Clears 6 and 7 were tested for this specification, and an examination of Figures 4 to 8 will show at once the viscosity and rate of withdrawal necessary for the desired film thickness. A series of films could be prepared by the dip coater covering a range of film thickness for a study of variation of film properties with thickness. In this way the optimum film thickness could be determined.

This work was done under conditions of constant temperature and humidity (78° F., 50 per cent relative humidity). It is well known that viscosity changes appreciably with temperature, and the temperature coefficient is not the same for a variety of coating materials. Where constant temperature is not available, it would be desirable to determine the viscosity of the coating under the same conditions as application and immediately before using. The rate of evaporation of the solvent from the film and consequently the setting time of the film is affected by the humidity of the atmosphere, but this effect is not so great as the temperature-viscosity factor and may be neglected over the normal humidity changes.

Summary

Surface coating films of uniform thickness may be made by the dip coater on steel panels for test purposes.

Film thickness may be varied over the normal useful range by changing the viscosity of the coating, the rate of withdrawal, or both. The range of rate of withdrawal for a wide variety of surface coatings is from 2 to 4 inches per minute.

Viscosity is more important than per cent solids in determining film thickness at a given rate of withdrawal.

There is an optimum flowing quality for the production of uniform films over the normal thickness range. Coating materials with very poor flowing quality are not satisfactory for application by the dip method.

The data obtained indicate the range of viscosity and rate of withdrawal necessary for surface coatings suitable for dip application to give a desired film thickness. The actual conditions of application should be determined for any specific coating and film thickness.

Acknowledgment

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Effect of Ethanol Concentration on Purity of Potassium Chloroplatinate

In Determination of Potash in Fertilizers

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FOR some time the question of the proper concentration of ethanol to be used for the determination of potash in fertilizers has been under consideration.

Ford and Hughes (*2*) reported that higher potash values were obtained with 95 per cent ethanol than when 80 per cent ethanol was employed as directed by the A. O. A. C. method for potash in fertilizers (*1*). Since this work was published, it has been suggested that perhaps the higher values were due to differences in the composition of the precipitate of potassium chloroplatinate rather than to decreased solubility of potassium chloroplatinate in the higher percentage of the ethanol. It was the purpose of this work to investigate the possibility of changes in the composition of the potassium chloroplatinate precipitate when various concentrations of ethanol are used.

Table I. Effect of Ethanol Concentration on Potash Value of a Potassium Chloride Solution

Ethanol Concentration	K_2PtCl_6[a]
%	*Gram*
80	0.2017
85	0.2034
90	0.2047
95	0.2052

[a] Averages of 8 determinations.

Procedure

The work of Ford and Hughes was extended to include potash determinations using 85 and 90 per cent as well as 80 and 95 per cent ethanol and acid-ethanol. These determinations were made on a solution of pure potassium chloride without evaporation and ignition. The temperature (20° C.) and the volume of ethanol (160 ml.) used were kept constant in all cases. The results obtained are presented in Table I.

Sufficient quantities of potassium chloroplatinate were prepared as above, using the four concentrations of ethanol, so that approximately 1 gram of each could be dissolved in water to make 500 ml. of solution, and 25-ml. portions were placed in weighed platinum dishes. Platinum reduction was accomplished by a modification of the method proposed by Swisher and Hummel (*3*) by adding 1 ml. of formic acid to each dish, heating to boiling, and transferring to a steam bath for 10 minutes. The solutions were filtered through weighed Gooch crucibles without attempting to remove all the platinum black from the dishes. The crucibles had previously been padded with a small disk of Whatman No. 5 filter paper and then with asbestos. After drying at 110° C., both the dishes and the crucibles were reweighed.

The filtrates from the platinum determination were acidified with nitric acid and chloride was determined gravimetrically by precipitating with silver nitrate. The platinum and chloride values are presented in Table II.

Table II. Effect of Ethanol Concentration on Composition of Potassium Chloroplatinate Precipitate

Ethanol Concentration	Platinum			Chloride		
	Found	Theoretical	Difference	Found	Theoretical	Difference
%	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
80	0.0200	0.0201	0.0001	0.0215	0.0219	0.0004
85	0.0200	0.0201	0.0001	0.0215	0.0219	0.0004
90	0.0199	0.0201	0.0002	0.0216	0.0219	0.0003
95	0.0201	0.0201	0.0000	0.0216	0.0219	0.0003

Discussion

Table I is further confirmation of the findings of Ford and Hughes that higher percentages of ethanol give higher potash values.

Table II indicates that, within experimental error, the composition of the potassium chloroplatinate precipitate is not changed by varying the concentration of the ethanol used.

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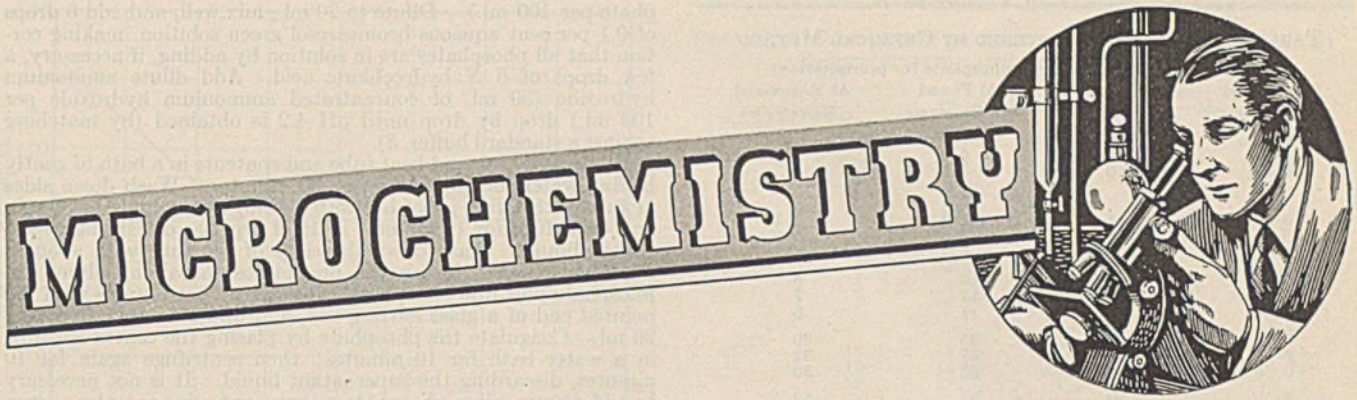

Determination of Aluminum in Biological Material

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THE application of a spectrochemical method to the determination of aluminum in biological material (*5*) yielded results which were often lower than those previously reported by many other workers (*14*). A careful study of the method resulted not only in a number of modifications which greatly increased the precision of analysis in the lower range of concentrations but also in the development of a chemical method which could be used to check the spectrographic findings. The refinements incorporated in the spectrochemical method and the details of the chemical method employed in testing it are described in the following paragraphs.

Spectrochemical Method

The chief difficulty in the use of the spectrochemical method was encountered in its application to extremely low levels of concentration where precision is affected by background fog and residual aluminum in the reagents and electrodes. The residual aluminum even in the case of individual electrodes may vary considerably and therefore a blank correction cannot be used with any degree of safety. While a number of earlier batches of electrodes were satisfactorily purified by means of prolonged acid extraction (*6*), this treatment has not proved consistently reliable for more recently purchased batches. This fact led to an examination of available purified electrodes and to the choice of 0.6-cm. (0.25-inch) Dow purified graphite electrodes for this work.

The reagents to be employed are purified either by redistillation (distilled water, nitric and hydrochloric acids) or, when possible, by the removal of aluminum as the phosphate (at pH 4.2). The latter method has proved satisfactory for the purification of the spectroscopic base (*4*) which is used to derive the calibration curves and as the diluent added to the prepared samples to control their inorganic salt composition (*4*). The small quantities of residual aluminum which, together with plate emulsion fog, affect the reliability of the lower sections of the working curves may be determined spectrographically when the working curves are derived (*7*).

The reliable evaluation of photometric data obtained for lines, the intensities of which are just above that of the emulsion fog, has lately been the subject of a number of papers (*7, 15, 17, 20*). The method used to correct for background fog in this study is fundamentally the same as that described by Pierce and Nachtrieb (*17*), with the additional precaution that the internal standard employed is a line in the same range of intensity as the test line (*7*). The advantage of such a procedure is obvious from the fact that the values for the density or intensity of lines in the medium and upper blackening levels suffer little change due to background. With 15 mg. of bismuth per 100 ml. of prepared test solution (0.2 ml. of which are placed on the arc) the bismuth lines at 2993.4 and 3024 are of suitable intensity to serve as standard lines for the evaluation of aluminum (3082 Å.) in the respective ranges of from 0.01 to 0.10 mg. per 100 ml., and from 0.05 to 2.00 mg. per 100 ml., of the prepared sample.

The nitric acid solutions (plus hydrochloric acid, if tin is present) of the dry-ashed (500° C.) samples are adjusted to volumes dictated by experience. Generally the solutions of ashed solid tissues, blood, and individual food items are adjusted to volumes corresponding to the weight in grams of the original material. The solutions of ashed urine samples are made up so that each milliliter corresponds to 10 ml. of fresh urine, those of feces ash to a total volume of 100 ml., and the solutions of ashed mixed food samples to 500 ml.

In the case of urine the internal standard (1 ml. of a solution containing 1.5 mg. of bismuth) is included in making the final volume adjustment. Other materials are prepared for spectrographic analysis by mixing—in graduated 15-ml. Pyrex centrifuge tubes—suitable quantities of the prepared sample solutions and definite amounts of a spectroscopic buffer salt solution containing the proper amount of the internal standard (15 mg. of bismuth per 100 ml. of buffer solution). (The buffer solution or diluent contains the inorganic salts of ashed urine and is adjusted in volume so that 1 ml. is equivalent to 10 ml. of normal urine, *4*.) The mixtures are concentrated in a glycerol or oil bath to the volumes of diluent employed, and 0.2-ml. portions are dried in the craters of graphite rods (3.75 cm., 1.5 inch, in length and 0.6 cm., 0.25 inch, in diameter). The dried rods are then burned (with similar untreated rods serving as negative electrodes) for 2 minutes as a direct current arc from a 120-volt line at 10 amperes and with a 50-volt drop across the electrodes. The craters (10 mm. in depth and 3 mm. in diameter) in the two electrodes help to improve the steadiness of the arc. Step spectra are obtained by rotating a 5-step sector (factor of 2) before the slit of a Bausch & Lomb large quartz Littrow spectrograph (set at 5) and photographing the refracted light on Eastman No. 33 plates. The step densities for the test and standard lines are measured in a nonrecording densitometer and the separation between the respective Hurter-Driffield curves (plotted as densities against the log relative exposures of the steps) at a constant density (0.30) gives the aluminum concentration when read from a calibration curve derived by adding known amounts of aluminum to the spectroscopic buffer (*6*). In the case of very weak lines (0.01 to 0.10 mg. of aluminum per 100 ml. of solution), only the maximum exposure steps are used, as described in a recent paper (*7*). In this case it is advisable to correct for background fog, and with urine samples to correct as well for any residual aluminum which may have been present in the salt stock used to derive the working curve (Figure 1 and *7*).

TABLE I. ALUMINUM RECOVERED BY CHEMICAL METHOD

(Using 200 mg. of diammonium phosphate for precipitation)

Range Used	Al Added	Al Found	Al Recovered
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*
0–5	0	5.0	..
0–5	0	5.2	..
0–5	0	4.9	..
		Av. 5.0	
0–5	1	6.3	1.3
0–5	1	5.9	0.9
0–5	1	6.1	1.1
0–50	5	10	5
0–50	5	12	7
0–50	5	11	6
0–50	30	35	30
0–50	30	37	32
0–50	30	35	30
0–50	50	57	52
0–50	50	54	49
0–50	50	55	50

Chemical Method

None of the available chemical methods was adequate for the authors' purpose, since they needed a method which could be applied over a wide range of values (1 microgram to several hundred milligrams).

Schmidt and Hoagland's aluminum phosphate method (*19*) is excellent for gravimetric work but obviously could not be used for minute quantities. The available micromethods employing 8-hydroxyquinoline (oxine), the ammonium salt of aurin tricarboxylic acid (aluminon), or Alizarin Red S, were not suitable because of losses and manipulative difficulties.

The oxine methods as described by Berg (*3*), Hahn and coworkers (*10, 11*), and Schams (*18*) were time-consuming and inadequate for amounts less than 50 micrograms of aluminum. Quantities below this value did not precipitate readily as the oxinate, a fact which Alten and co-workers had reported (*1*). In addition, the many manipulative steps entailed unavoidable mechanical losses. The color obtained by coupling the diazotized oxine (liberated from the aluminum oxinate) with sulfanilamide was photometrically suitable, however, and therefore the method could readily be applied to quantities of aluminum above 50 micrograms.

The aluminon method has been employed by a number of investigators (*9, 12, 16, 22–25*). That described by Cox (*9*) was chosen as the best representative of these methods, but was found to be unsatisfactory. The principal difficulties encountered were loss of aluminum through adsorption or occlusion when interfering iron was removed as the hydroxide, interference by the phosphate ion in the final estimation, and failure of the color to obey Beer's law.

The Alizarin Red S method, first described by Atack (*2*), then carefully studied by Yoe and Hill (*26*) and applied by Underhill and Peterman (*21*), appeared to be the method best suited to the authors' purpose. With certain modifications which are included in the description below, it has the following advantages over others using Alizarin Red S: The isolation of aluminum as the phosphate followed by washing with water (and the subsequent removal of iron with cupferron) removes interfering substances which affect the final color (*2*); employment of a mixed color makes it possible to avoid the use of acetic acid, which attacks the aluminum lake (*2*); and this colorimetric procedure permits the use of a single stable calibration curve which obeys Beer's law.

Method of Procedure

ISOLATION OF ALUMINUM AS ALUMINUM PHOSPHATE. Samples of urine (100 ml. or less), blood (5 grams or less), tissues (5 to 100 grams), feces (24-hour excretion), and mixed food are prepared for analysis by the dry-ashing method used for spectrographic work (*4*).

Transfer the entire prepared sample of urine or blood—or a suitable aliquot in case the aluminum content is greater than 50 micrograms—to a 50-ml. graduated conical centrifuge tube. Add 1 mg. of iron (100 mg. of iron as ferric chloride per 100 ml. of water), 1 ml. of saturated ammonium acetate solution, and 200 mg. of diammonium phosphate (4 grams of diammonium phosphate per 100 ml.). Dilute to 20 ml., mix well, and add 6 drops of 0.1 per cent aqueous bromocresol green solution, making certain that all phosphates are in solution by adding, if necessary, a few drops of 6 *N* hydrochloric acid. Add dilute ammonium hydroxide (30 ml. of concentrated ammonium hydroxide per 100 ml.) drop by drop until pH 4.2 is obtained (by matching against a standard buffer, *8*).

Dilute to 30 ml. and heat tube and contents in a bath of gently boiling water for approximately 30 minutes. Wash down sides of tube with a fine jet of hot water, bring the volume to 30 ml., and centrifuge for 10 minutes at 1800 r. p. m., discarding supernatant liquid. Wash down the sides of the tube with about 2 ml. of hot water, loosen the precipitate containing aluminum phosphate plus iron phosphate, breaking up hard lumps with the pointed end of a glass stirring rod, and dilute to approximately 20 ml. Coagulate the phosphate by placing the centrifuge tube in a water bath for 10 minutes; then centrifuge again for 10 minutes, discarding the supernatant liquid. It is not necessary to add diammonium phosphate to prepared urine samples or iron to prepared blood samples. Moreover, in the case of blood the amount of phosphate normally present permits satisfactory precipitation of aluminum, if only 100 mg. of the diammonium phosphate are added.

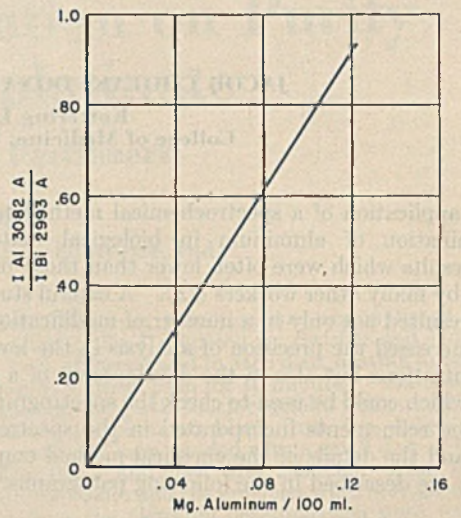

FIGURE 1. CALIBRATION CURVE FOR SPECTROGRAPHIC DETERMINATION OF MICRO QUANTITIES OF ALUMINUM

Corrected for background and residual aluminum

REMOVAL OF IRON INTERFERENCE. Dissolve the precipitate with 5 ml. of dilute sulfuric acid (10 ml. of concentrated sulfuric acid per 100 ml.), place in the hot-water bath to effect solution, dilute to approximately 10 ml., and centrifuge for 10 minutes to separate silica. Transfer the supernatant liquid to a 150-ml. Squibb type separatory funnel and dilute to 20 ml. Add 2 ml. of a cold aqueous cupferron solution (6 grams per 100 ml.) and allow to stand for 1 minute after thorough shaking. Add 10 ml. of a water-saturated benzene-ether mixture (equal parts by volume), shake for 1 minute, and allow complete separation of the two phases. Remove the aqueous layer to a second separatory funnel and repeat treatment with 10 ml. of the benzene-ether mixture. Remove the aqueous fraction to a 30-ml. Kjeldahl flask containing 1 ml. of concentrated nitric acid, add one or two small clean Carborundum bumping stones, and heat with a microburner until fumes of sulfur trioxide appear. Cool, add 1 ml. of water, and again heat until appearance of sulfur trioxide fumes, to expel oxides of nitrogen.

PHOTOMETRIC MEASUREMENT OF THE MIXED COLOR OF THE ALUMINUM LAKE OF ALIZARIN RED S PLUS EXCESS ALIZARIN RED S. If the range of 0 to 5 micrograms of aluminum is to be used, rinse the contents of the 30-ml. Kjeldahl flask completely into a 25-ml. glass-stoppered cylinder. Dilute to 14 ml., add 1 ml. of an aqueous solution of Alizarin Red S (0.075 gram of Alizarin Red S per 100 ml.), then add 10 ml. of dilute ammonium hydroxide (30 ml. of concentrated ammonium hydroxide per 100 ml.), mix the contents, and measure the density immediately at 580 mμ, with a suitable spectrophotometer.

If the range is 0 to 50 micrograms of aluminum, use a 100-ml. glass-stoppered cylinder. Dilute to 85 ml., and add 5 ml. of

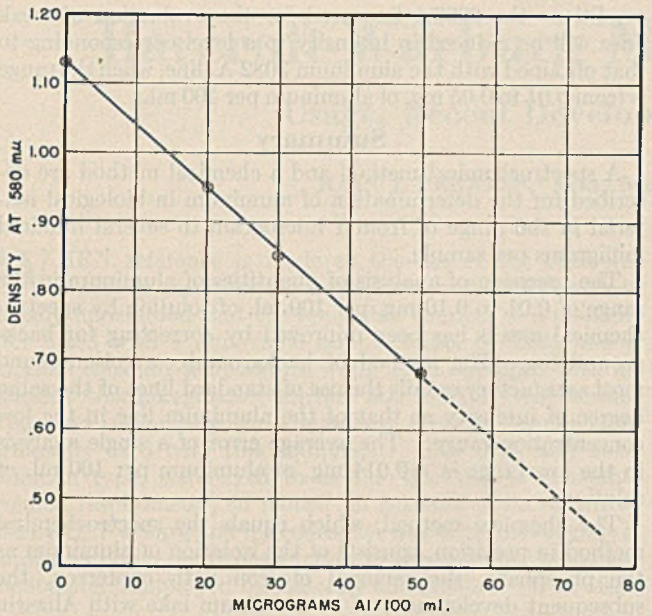

FIGURE 2. CALIBRATION CURVE FOR SPECTROPHOTOMETRIC MEASUREMENT OF MIXED COLOR
Cell length 2.5 cm.

the same Alizarin Red S solution and 10 ml. of dilute ammonium hydroxide. Other procedures are the same.

The density curve as shown in Figure 2 for the range 0 to 50 micrograms was obtained with a pure aqueous solution of aluminum chloride, containing 5 ml. of dilute sulfuric acid (10 ml. of concentrated sulfuric acid per 100 ml.). Density measurements were obtained with the photoelectric spectrophotometer described in a previous paper (*13*). A reagent blank of approximately 5 micrograms of aluminum is obtained when 200 mg. of diammonium phosphate are used.

Results

Table I lists the recoveries obtained by the chemical method when known amounts of aluminum were added and 200 mg. of diammonium phosphate were used for precipitation of aluminum. The agreement between findings obtained by the chemical and the spectrographic methods is shown in Tables II, III, and IV. Table II gives recoveries of known amounts of added aluminum approximating those which may be encountered in fecal and mixed food samples. Table III illustrates the agreement between the results obtained by the two methods at the lower levels of concentration. These results, listed in the frequencies of their occurrence, with their calculated mean values, were obtained from duplicate samples of consecutive daily urinary specimens from a subject under study. The range of application of the two methods, the agreement in the findings obtained by their use, and also the levels of aluminum concentration which may be encountered in miscellaneous material may be seen in Table IV.

Discussion

Aluminum is the most widely distributed of the metals, forming as it does about 7 per cent of the earth's surface. Contamination, therefore, is a serious factor in the determination of aluminum and its complete avoidance is practically impossible. Nevertheless, the consistently low results and the close agreement between the means obtained on independent aliquots of urine samples by two such distinctly different methods (Table III) point to the fact that contamination has been held to a minimum. The effect of adventitious aluminum depends, of course, on the range of concentrations

TABLE II. COMPARISON OF ANALYTICAL RESULTS OBTAINED BY SPECTROGRAPHIC AND CHEMICAL METHODS

Feces Sample	Al Added	Chemical Method Al found	Chemical Method Al recovered	Spectrographic Method Al found	Spectrographic Method Al recovered
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0721	0	1.66	..	1.84	..
	0	1.66	..	1.80	..
	0	1.40	..	1.64	..
	Av.	1.57		Av. 1.76	
0721	5	6.66	5.09	6.50	4.74
	5	6.50	4.93	7.25	5.49
	5	6.16	4.59	6.75	4.99
		Av.	4.87	Av.	5.07
0721	10	12.00	10.43	11.20	9.44
	10	12.25	10.68	11.30	9.54
	10	10.75	9.18	12.50	10.74
		Av.	10.07	Av.	9.90
0721	100	105.0	103.4	114.8	113.0
	100	105.0	103.4	109.8	108.0
	100	92.5	90.9	101.8	100.0
		Av.	99.2	Av.	107.0

TABLE III. DETERMINATION OF ALUMINUM IN CONSECUTIVE SAMPLES OF URINE FROM AN EXPERIMENTAL SUBJECT

(Comparison of spectrographic and chemical results)

Al Found, Mg./l.	Frequencies of Occurrence Chemical	Spectrographic
0.010–0.0149	1	..
0.015–0.0199	2	..
0.020–0.0249	7	..
0.025–0.0299	4	..
0.030–0.0349	7	4
0.035–0.0399	13	8
0.040–0.0449	9	25
0.045–0.0499	12	10
0.050–0.0549	13	23
0.055–0.0599	6	4
0.060–0.0649	4	9
0.065–0.0699	4	3
0.070–0.0749	5	2
0.075–0.0799	0	0
0.080–0.0849	4	3
Totals	91	91
Mean	0.046	0.050
Probable error	±0.001	±0.001
Std. deviation	±0.016	±0.011
Coefficient of variation	34.59%	21.75%

encountered and may be serious in the case of urine, blood, or animal tissues with a low aluminum content. It is therefore advisable to carry out analyses in duplicate whenever sufficient material is available.

Silica does not interfere with the determination and there is no evidence that any considerable portion of the aluminum present in prepared samples of feces or mixed food is in the form of insoluble silicate. A number of tests on such material containing 1.5 to 100 mg. of aluminum showed that 99 per cent or more of the aluminum was in the soluble form. The fact that in one case 1 per cent of the aluminum was found in the silica does not mean that the aluminum was present as an insoluble silicate; a more probable explanation is that it was difficult to remove small amounts of adsorbed aluminum completely by washing. Silica is removed in the chemical method for photometric reasons only.

At least 1 mg. of iron was found to be essential for the complete adsorption or entrainment of 50 to 75 micrograms of aluminum as the phosphate. Higher amounts are unnecessary, and were avoided because of the increased manipulation required in washing the phosphate and in removing the iron with cupferron. Phosphate must be present in an amount in excess of that needed to combine with all of the added iron and the aluminum. Best results were obtained when 200 mg. of diammonium phosphate were employed. Larger amounts give rise to high blanks, and, in addition, increased quantities lead to incomplete removal of excess diammonium phosphate by the washing process. The phosphate remaining, when carried through to the estimation step, may cause a partial precipitation of aluminum phosphate and thus produce low

Table IV. Comparison of Analytical Results Obtained by Spectrographic and Chemical Methods

Sample	Aluminum Found: Spectrographic	Chemical
	Mg./l.	*Mg./l.*
Urine 803	0.04	0.03
Urine 1483	0.06	0.06
Urine 1495	0.08	0.07
	Mg./100 g.	*Mg./100 g.*
Blood 606	0.02	0.03
Blood 843	0.03	0.03
Blood 945	0.03	0.03
	Mg./24 hours	*Mg./24 hours*
Feces 3496[a]	338	332
Feces 4188	12.5	12.6
Food 3145[a]	330	324
Food 3717	3.6	3.9

[a] Following addition of Al to diet.

results. This fact also limits the upper range of estimation by chemical means to 50 or 75 micrograms per aliquot, since larger quantities of aluminum also tend to recombine with the phosphate under the conditions of this method.

The only difficulty encountered with the chemical method as outlined in this paper is in establishing the range of the concentration of aluminum in the specific sample; this can be done only on the basis of experience with a particular series of samples. In the case of urine and blood samples, the aliquots designated usually can be analyzed with the 0 to 5-microgram curve, with extrapolation to 7.5 micrograms if necessary. This procedure is preferable to using a 0 to 10 microgram range, since the quantities of alizarin required in this case give an initially high density and thus make it difficult to evaluate small color changes. However, since the method is sensitive, small aliquots can be used, and the analysis can be repeated with larger aliquots when this is deemed advisable.

The stability of the lake formed is sufficient for rapid measurement—within 10 to 15 minutes. Under the conditions outlined the densities of the mixed color (with increased aluminum) follow Beer's law (Figure 2), the greatest density spread between the aluminum-lake color and the excess Alizarin Red S color being observed at 580 mμ. For most accurate results each sample should be developed immediately before reading.

With the spectrographic method there is no question of specificity when an instrument of sufficient dispersion (large quartz Littrow type) is employed. The problem here, as previously pointed out, is one of reproducibility, particularly for the extremely low concentration ranges. The reproducibility obtainable by the present method is excellent when judged by the variations in individual results used to plot Figure 1. A varying number of spectra was employed for each point. Table V shows number of spectra and mean error of a single analysis as read from the finally derived curve, plotted from the mean values at a number of concentrations.

In the higher concentrations the average error of spectrochemical analysis is approximately ± 10 per cent. The evaluation of spectrum-line blackenings by the usual interval of log *E* separation (*6*, *7*) may be used with excellent results for quantities above 0.05 mg. of aluminum per 100 ml. of solution. The older method of evaluating weak spectrum lines by the method of opacity separation (*6*, *7*) may also be used when background is present, provided the standards used to derive the curve had approximately the same intensity of background. This older method fails particularly in dealing with very weak lines in spectra having practically no background. In the latter case, the separations are too large, and consequently they yield low results. Although the bismuth 3024 line has been used as an internal standard it is also possible to use the 2898 line (*7*). When this is done the amount of bismuth added should be reduced to 5 mg. per 100 ml. Under these conditions the 2993.4 line used for the evaluation of weak lines will be reduced in intensity to a level corresponding to that obtained with the aluminum 3082 Å. line, when the range is from 0.01 to 0.05 mg. of aluminum per 100 ml.

Summary

A spectrochemical method and a chemical method are described for the determination of aluminum in biological material in the range of from 1 microgram to several hundred milligrams per sample.

The precision of analysis of quantities of aluminum in the range of 0.01 to 0.10 mg. per 100 ml. of solution by spectrochemical means has been improved by correcting for background fog. The method of background correction found most satisfactory entails the use of standard lines of the same degree of intensity as that of the aluminum line in the low concentration range. The average error of a single analysis in the low range is ±0.014 mg. of aluminum per 100 ml. of solution.

The chemical method, which equals the spectrochemical method in precision, consists of the isolation of aluminum as the phosphate, the removal of iron with cupferron, the subsequent development of an aluminum lake with Alizarin Red S, and the measurement of the mixed color by means of a photoelectric spectrophotometer.

Table V. Reproducibility of Findings by Spectrographic Means

(Low concentration range)

Concentration *Mg./100 ml.*	Spectra	Mean Error of Single Analysis *Mg./100 ml.*
0.03	6	±0.012
0.04	6	±0.010
0.08	11	±0.015
0.13	10	±0.019

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Barcroft-Warburg Manometric Apparatus

Usage, Recent Developments, and Applications

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WHEN reference is made to the use of the Barcroft-Warburg respirometer in experimental methods it is usually understood that the apparatus consists of a manometer fitted with a removable sample vessel or flask, and a suitable constant-temperature water bath equipped with an efficient thermoregulator, stirrer, and shaking mechanism. There are essentially three standard types of manometers ordinarily used with this equipment. The first, and most common type, is referred to as the Warburg or constant-volume respirometer, so named on account of its extensive use by O. Warburg and his colleagues at Berlin, although it is similar in design and construction to the original "blood gas manometer" employed by Barcroft and Haldane (*6*) in 1902.

Constant-Volume Respirometer

The Warburg manometer, as illustrated in Figure 1, consists of a Pyrex U-tube with an outside diameter of 7 to 8 mm., carefully selected for uniformity and precision of bore. For proper sensitivity, the internal cross section of the bore should be approximately 1 sq. mm. The vertical arms of the tube are accurately graduated in millimeters from 0 to 30 cm., and the horizontal alignment of the graduations is of the order of 0.1 mm. At the base of the manometer a rubber reservoir and screw-clamp arrangement are attached, by which the level of the indicating liquid in the tube may be adjusted. One end of the U-tube is open to the air and the other end, as shown, is constructed with a horizontal arm for attaching a suitable vessel by means of a standard taper (ⅎ 7/25) interchangeable ground-glass joint. A three-way stopcock not only permits communication with the air but also serves for the introduction of different gas mixtures into the respiring material, dependent upon the experimental requirements.

Each manometer of this type is equipped with a removable support or backing made of Monel metal or wood and also a special sleeve for attaching to the shaking mechanism of the thermostat as described below. Such an arrangement has been found necessary in view of the fact that when the manometer is in its proper position the sample vessel must be completely immersed in the constant-temperature water bath.

Before making a reading on the Warburg type of manometer, the level of the indicating fluid in that arm of the U-tube connecting with the test vessel is always adjusted by means of the thumbscrew device to the same given point—for example, the 150-mm. mark. Readings are made on that arm of the manometer which is open to the air only, and the difference of pressure between the interior of the vessel and the outer air is thus obtained by subtracting 150 from these readings. From the reading of the manometer the volumes of gas absorbed or evolved by the respiring material can be calculated according to the following formula:

$$x = h\left[\frac{V_g\frac{273}{T} + V_f(\alpha)}{P_0}\right] \qquad (1)$$

where

x = cu. mm. of gas at standard temperature and pressure

h = reading of manometer (change in height in manometric graduations)

V_g = free volume of gas in the vessel and manometer to the level of manometric fluid (total volume of apparatus less volume of sample, liquids, and detachable supports placed in the vessel)

T = absolute temperature of the water bath surrounding the vessel

V_f = volume of all liquids in the vessel in which the measured gas might dissolve

α = solubility of the gas being measured in the vessel liquid at temperature T (see Bunsen's solubility table)

P_0 = normal pressure in terms of manometric fluid (for Brodie's solution, 10,000 mm.). Brodie's solution may be prepared as follows: sodium chloride, 23 grams; sodium tauroglycocholate, 5 grams; water to 500 ml. (10,000 mm. of this solution are approximately equivalent to 760 mm. of mercury).

In experiments where a constant volume of material is to be used, the quantity within the brackets in Formula 1 remains constant for a given vessel and is referred to as the vessel constant, k. The majority of vessels used with the Warburg respirometer possess volumes of approximately 15 ml. and their respective constants usually range from 1 to 3. Therefore, if the vessel constant is known, it is only necessary to multiply the manometer reading by it to find the amount of gas evolved at standard temperature and pressure.

MacLeod and Summerson (*55*) have recently described a graphic method for determining Warburg vessel constants at various fluid volumes. This type of procedure eliminates the tedious computations and is, no doubt, conducive to greater accuracy, since a linear relationship exists between the vessel

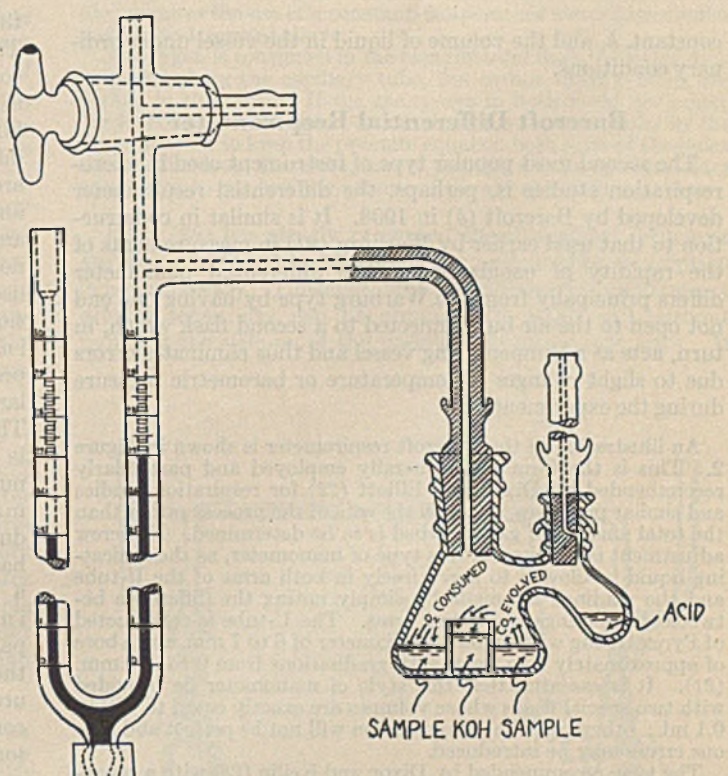

FIGURE 1. WARBURG CONSTANT-VOLUME MANOMETER AND REACTION VESSEL

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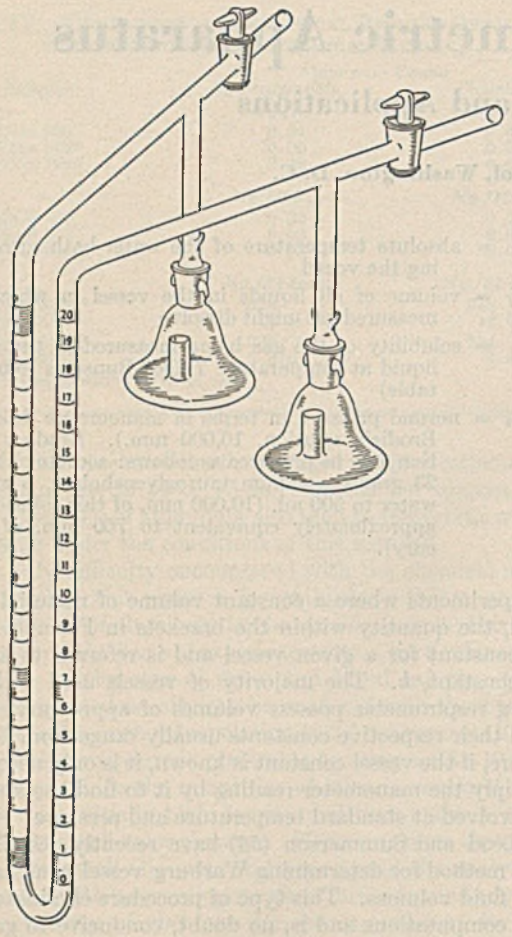

FIGURE 2. BARCROFT DIFFERENTIAL MANOMETER WITH FLASKS

constant, k, and the volume of liquid in the vessel under ordinary conditions.

Barcroft Differential Respirometer

The second most popular type of instrument used in microrespiration studies is, perhaps, the differential respirometer developed by Barcroft (*5*) in 1908. It is similar in construction to that used earlier by Warburg (*91*) in measurements of the rapidity of ozonization. The differential manometer differs principally from the Warburg type by having one end not open to the air but connected to a second flask which, in turn, acts as a compensating vessel and thus eliminates errors due to slight changes of temperature or barometric pressure during the experiment.

An illustration of the Barcroft respirometer is shown in Figure 2. This is the form now generally employed and particularly recommended by Dixon and Elliott (*22*) for respiration studies and similar processes, in which the rate of the process rather than the total amount of gas absorbed is to be determined. No screw adjustment is required in this type of manometer, as the indicating liquid is allowed to move freely in both arms of the U-tube and the readings are made by simply noting the difference between the readings of the two arms. The U-tube is constructed of Pyrex tubing with an outside diameter of 6 to 7 mm. and a bore of approximately 2 sq. mm. with graduations from 0 to 200 mm. (*21*). It is essential that this style of manometer be provided with two special flasks whose volumes are exactly equal to within 0.1 ml.; otherwise, the compensation will not be perfect and serious errors may be introduced.

The type recommended by Dixon and Keilin (*23*) with a capacity of about 40 ml., has been found very satisfactory, since it is made with a removable side arm and hollow stopcock for introducing acid or alkali during an experiment. This style of flask is shown in Figure 3. In this type of manometer also it is necessary to determine the apparatus constant, k, which may be derived from the following simplified formula:

$$x = h\left(\frac{A}{2}\frac{P_0}{V_0'}\right)\left(\frac{V_g\frac{273}{T} + V_f\,\alpha}{P_0} + \frac{A}{2}\frac{273}{T}\right) \qquad (2)$$

where

x = cu. mm. of gas evolved in reaction vessel (right flask)
h = reading of manometer
A = area of bore in manometer (sq. mm.)
V_0' = volume of gas space in compensating (left) flask, including manometer tubes to the level of manometric fluid, normally kept at 100-mm. graduation
V_g = volume of gas space in reaction vessel (right flask)
T = absolute temperature of water bath
α = solubility of gas being measured in vessel liquid at temperature T
V_f = volume of all liquids in the vessel in which the measured gas might dissolve
P_0 = normal pressure in terms of manometric fluid

The Barcroft respirometer as modified by Dixon and Elliott (*22*) is preferred by a great many workers for measurements involving the oxygen uptake, the respiratory carbon dioxide production, and glycolysis of tissue slices or homogenized tissue suspensions (*26*, *62*, *77*, *102*).

Summerson Manometer

The third style of manometer in current use with the Barcroft-Warburg apparatus is an ingenious device recently developed by Summerson (*83*) and illustrated in Figure 4. It offers in a single apparatus the possibilities of either direct or differential manometric measurements. For direct measurements it is the equivalent of two single manometers of the conventional Warburg type in which one arm of the manometer is open to the atmosphere. When used as a differential manometer it operates at constant volume, with the attendant simplification of differential measurement made possible by use of constant-volume vessel constants.

Readings on the Summerson manometer are made along the two inner arms which are graduated from 0 to 450 mm. The manometer fluid levels and vessel volumes are maintained constant at the calibration marks on the outer arms by the usual method of adjusting the pressure screw device on the fluid reservoir. The differential readings are equally precise at any vessel pressure, and a sensitive manometer fluid may be used for the determination of small differences in pressure in the presence of relatively large total pressures. This type of manometer is adaptable to a number of different manometric procedures, each of which has heretofore required a separate type of instrument. It is particularly suited to the differential measurement of the oxygen consumption, respiratory carbon dioxide output, and aerobic glycolysis in metabolism studies.

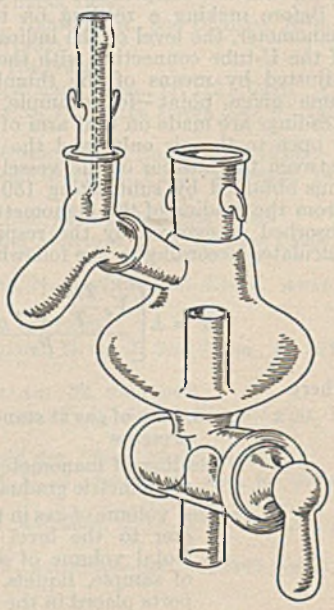

FIGURE 3. DIXON-KEILIN FLASK

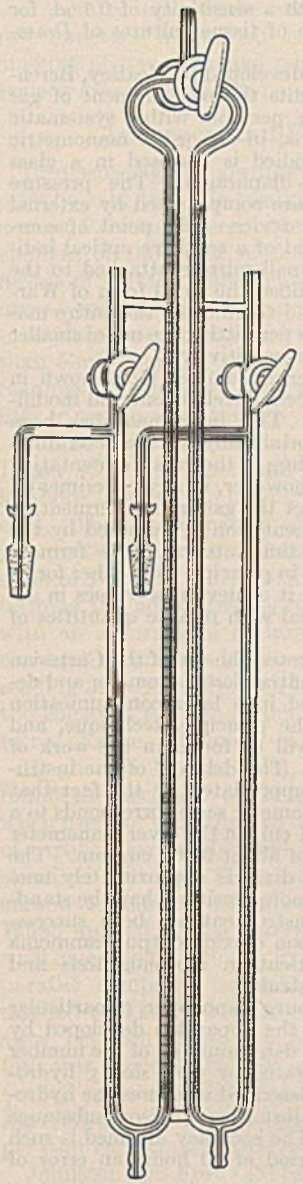

FIGURE 4. SUMMERSON COMBINATION MANOMETER

Constant-Temperature Bath

Close and reliable temperature control is of the utmost importance in the Barcroft-Warburg procedures, since the apparatus is arranged to measure kinetically the oxygen consumption as a function of time under conditions of constant temperature and atmospheric pressure. Precise temperature control of the bath or thermostat to ±0.02° C. or better is accomplished by means of an efficient metastatic mercury or bimetal thermoregulator, a sensitive relay, motor stirrer, and an immersion heater arranged to provide a symmetrical distribution of heat throughout the bath.

As shown in Figure 5, the shaking mechanism operating on ball-bearing rollers is an integral part of the assembly and permits the attached manometers to move through the bath with a horizontal reciprocating motion, thus allowing readings to be made without stopping the shaking action. By means of a rheostat control various speeds of shaking up to about 200 complete oscillations per minute with an adjustable amplitude of movement from 0 to 4 cm. can be obtained, and, providing the shaking mechanism is driven by a synchronous motor, any speed selected will be constant regardless of variations in load or line voltage. Dixon and Elliott (*22*) demonstrated that the rate of shaking is a very important factor, especially when studying the velocity of oxygen uptake of materials such as baker's yeast.

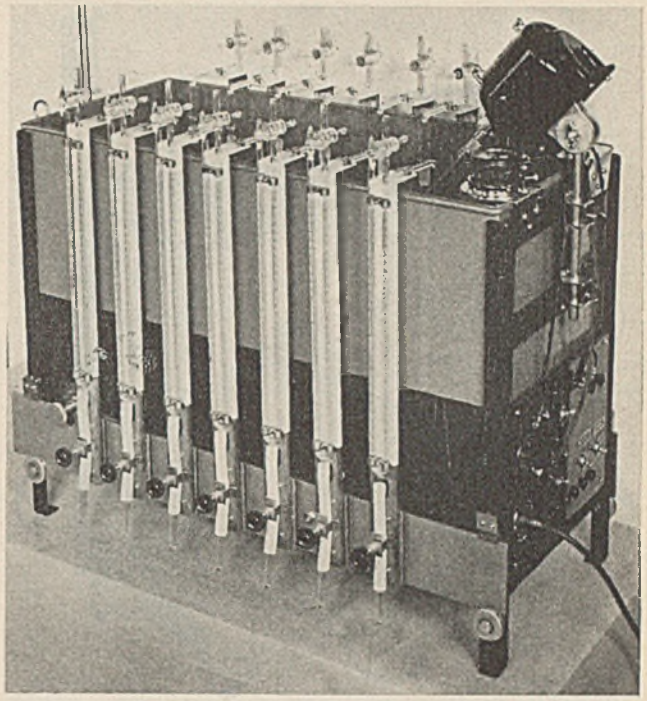

FIGURE 5. BARCROFT-WARBURG APPARATUS EQUIPPED WITH 14 MANOMETERS

Recent Developments and Modifications

One of the most widely used and versatile modifications of the Barcroft-Warburg apparatus is the differential volumeter devised independently by Fenn in 1927 (*28*) for the measurement of cellular respiration and other processes. This highly sensitive instrument is illustrated in Figure 6. It operates upon the volumetric rather than the manometric principle and is similar in construction to the differential volumeter first introduced by Thunberg in 1905 (*87*), and later modified by Winterstein (*99*) and Widmark (*98*).

The instrument consists essentially of a control flask and an experimental flask connected by a capillary tube containing an index drop of kerosene stained with Sudan III dye to improve its visibility. The capillary is constructed of precision-bore Pyrex tubing with an internal cross section of approximately 0.3 sq. mm. (to contain 3 cu. mm. per cm. of length), and graduations extending from 0 to 15 cm. in 1-mm. subdivisions. The experimental flask has two side arms situated 90° apart to facilitate the transfer of contents separately, and also a stopcock for the introduction of different gas mixtures if needed. Sodium hydroxide may be placed in one side arm to absorb carbon dioxide and the other may contain any reagent which it is desired to introduce during the experiment. The volume of each flask is approximately 15 ml., although flasks possessing smaller volumes can be used with a resulting increase in sensitivity. This instrument also requires the use of a constant-temperature water bath similar to the one described above.

As oxygen is consumed in the experimental flask the index drop is drawn along the capillary tube, the carbon dioxide being absorbed in the alkali. If the gas spaces in both flasks are equal, the loss of gas due to absorption in the one is shared equally by the other in order to keep the pressure equal on both sides of the index drop. The drop, therefore, moves through a volume of capillary equivalent to one half the volume of the gas absorbed.

Fenn (*29*) has clearly presented the theoretical principles governing volumeter measurements and further description is not necessary. However, with the differential volumeter a greater sensitivity can be obtained (to 0.001 μl.) without reducing the volume of the vessels to inconvenient sizes. It

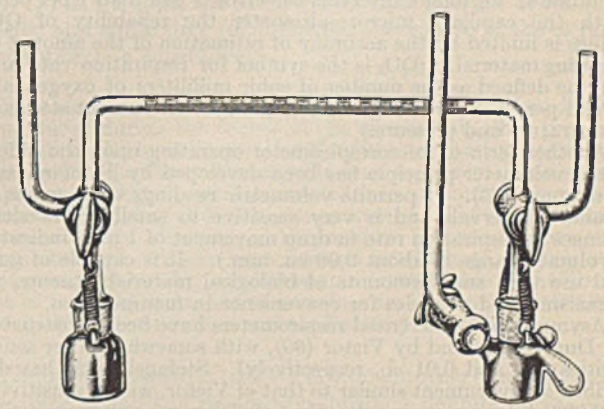

FIGURE 6. FENN DIFFERENTIAL VOLUMETER

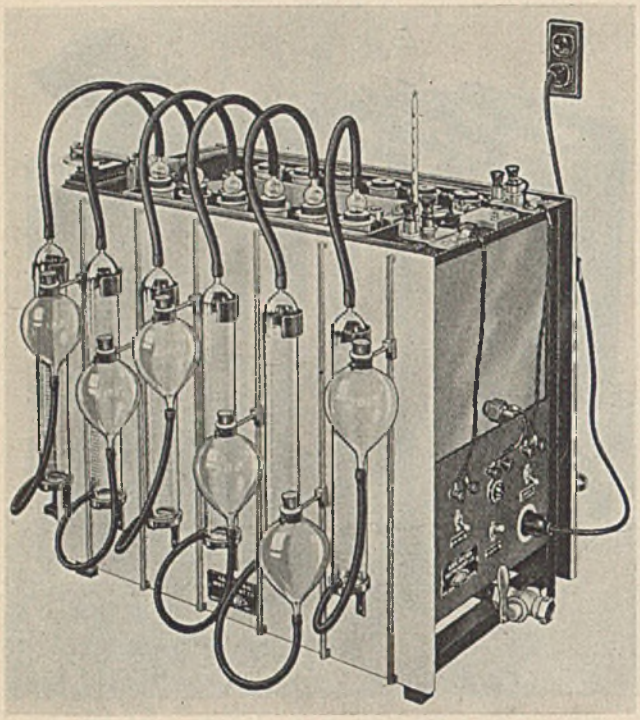

FIGURE 7. FLEISCHMANN-AMINCO FERMENTOMETER

is simple in operation, less expensive, more compact, and can be depended upon to yield results equal to the manometric methods in convenience and precision.

The subject of volumetric versus manometric methods for the determination of gas production has been discussed thoroughly by Eisenberg (*25*). Jares (*41*), Martin (*56*), and Burkholder (*16*) have used the differential volumeter for determinations of the respiratory quotient and oxygen consumption of certain bacteria and tissues. Recently Goodwin and Goddard (*34*) were highly successful in using the Fenn apparatus for measuring accurately the gaseous changes which take place between isolated woody tissue sections and their environment. They found the apparatus to be sensitive to 0.15 cu. mm. of gas per hour at 25° C. Schmitt (*69*) modified the differential volumeter for still higher sensitivity and applied it to the determination of the oxygen consumption of stimulated nerve. Gerard and Hartline (*32*) modified this instrument by taking advantage of the greater stability afforded by reducing the tissue chamber to capillary dimensions (0.5 to 1.2 mm. diameter) and placing this inside a relatively large "differential" chamber. Index drop movements, observed with an ocular micrometer, were consistent over 5-minute intervals, with volume changes of the order of 0.01 cu. mm.

A further development of the capillary method has recently been made by Tobias and Gerard (*88*), so that it is now convenient to follow the respiration of ten tissue samples at once, and it is possible to measure absolute gas volume changes of 0.001 cu. mm., minute by minute, with an error of some 8 per cent minute by minute; for longer intervals the error is less than 1 per cent. With the capillary microrespirometer the reliability of QO_2 values is limited by the accuracy of estimation of the amount of respiring material. (QO_2 is the symbol for respiration rate and may be defined as the number of cubic milliliters of oxygen absorbed per hour per milligram of dry might of tissue at standard temperature and pressure.)

Another form of microrespirometer operating upon the differential volumeter principle has been developed by Thimann and Commoner (*86*). It permits volumetric readings to be made at 1-minute intervals and is very sensitive to small or transient changes in respiration rate (a drop movement of 1 mm. indicates a volume change of about 0.06 cu. mm.). It is capable of general use with small amounts of biological materials, tissues, or organisms, and provides for convenience in manipulation.

Asymmetrical differential respirometers have been constructed by Duryee (*24*) and by Victor (*90*), with somewhat lower sensitivities (0.1 and 0.01 μl., respectively). Stefanelli (*82*) has described an instrument similar to that of Victor, with a sensitivity of 0.003 μl. for measuring the effect of cleavage on the respiration of a single egg of *Rana fusca*. Laser (*52*) and Meier (*57*) used a modified Warburg respirometer with a sensitivity of 0.1 μl. for the measurement of the respiration of tissue cultures of *Drosophila melanogaster*.

A novel type of respirometer was developed by Heatley, Berenblum, and Chain (*37*), which permits the measurement of gas exchanges of the order of 1 cu. mm. per hour with a systematic error not greater than that involved in the usual manometric methods. The material to be studied is enclosed in a glass chamber equipped with a mica diaphragm. The pressure changes taking place in the vessel are compensated by external pressure applied by a manometric device. The point of compensation is determined with the aid of a sensitive optical indicator system involving a pair of small mirrors attached to the mica diaphragm. Gerard (*31*) modified the usual form of Warburg apparatus by immersing in the thermostat the entire manometer including all gas space, thus permitting the use of smaller vessels with a consequent increase in sensitivity.

The new Fleischmann-Aminco fermentometer (*70*), shown in Figure 7, appears to be the most recent development and modification of the Warburg apparatus. This instrument has been used by a number of laboratories, principally for the determination of vitamin B_1 (thiamine) according to the yeast fermentation method (*71*, *72*). It may be used, however, in any experimental work in which it is desired to collect the gas from a fermenting solution (*73–75*). The rate of fermentation is measured by the amount of gas evolved in a given time interval. The fermentometer presents no new departures in principle from other forms of manometric apparatus, nor does it achieve new values in accuracy, sensitivity, or ability to deal with minute quantities of material.

Linderstrøm-Lang (*53*) first suggested the use of the Cartesian diver, illustrated in Figure 8, as an ultramicromanometer and details of the method were presented in a later communication (*54*). A thorough discussion of the principle, technique, and procedure for making the divers will be found in the work of Boell, Needham, and Rogers (*11*). The delicacy of the instrument and method can be readily appreciated by the fact that whereas 1 cm. on the Warburg manometer scale corresponds to a gas exchange of about 20 cu. mm., 1 cm. on the diver manometer scale corresponds to a gas change of about 0.008 cu. mm. The actual sensitivity of the Cartesian diver is approximately one-fifth of this value; it is 1500 times more sensitive than the standard Warburg manometer. This instrument has been successfully used for determining the carbon dioxide output, ammonia output, and the respiratory quotient in morphogenesis and metabolism studies of amphibian gastrula.

Another modification of the Warburg manometer, of particular interest to the organic chemist, is the apparatus developed by Kuhn and Moller (*50*) for the exact determination of the number of double bonds of highly unsaturated or very slowly hydrogenated substances. The method described measures the hydrogen used up by the substance against a comparison substance under exactly similar conditions. The accuracy attained is such that after hydrogenation for a period of 40 hours an error of ±0.5 per cent is not exceeded.

TABLE I. SENSITIVITY OF MICRORESPIROMETERS

Apparatus	Gas Change per Cm. Manometer Scale, Cu. Mm.	Author	Reference	Date
Standard Warburg manometer	20	Warburg	(*95*)	1926
		Dixon	(*20*)	1934
Warburg micromanometer	3–7	Laser	(*52*)	1932
		Meier	(*57*)	1931
Fenn volumeter	3–4	Fenn	(*28*)	1927
			(*29*)	1935
		Schmitt	(*69*)	1933
		Duryce	(*24*)	1936
Capillary micromanometer	1.8–2.3	Gerard and Hartline	(*32*)	1934
		Victor	(*90*)	1935
	0.06–0.12	Stefanelli	(*82*)	1937
	0.001 (sensitivity)	Tobias and Gerard	(*88*)	1941
Krogh-micromanometer	1.6	Bodine and Orr	(*10*)	1925
Differential volumeter	0.6	Thimann and Commoner	(*86*)	1940
Micromanometer with mica mirrors optically read	0.08–0.04	Heatley, Berenblum, and Chain	(*37*)	1939
Cartesian diver ultramicromanometer	0.008–0.02	Linderstrøm-Lang	(*53*)	1937
	0.001 (sensitivity)	Linderstrøm-Lang	(*54*)	1938
		Boell, Needham, and Rogers	(*11*)	1939

A comparison of the sensitivity of the various microrespirometers mentioned is presented in Table I. The amount of gas exchange per centimeter on the manometer scale is given for each type of apparatus.

Accuracy and Precision of Results

In discussing the accuracy of the Warburg techniques, it may be said that the readings are usually significant to the nearest 0.5 mm., thereby permitting the detection of volume changes of the order of 0.5 μl. (0.0005 ml.). However, by means of certain refinements and modifications, such as the use of special micromanometers with a fine capillary bore (less than 1 sq. mm.) and vessels of smaller capacity (less than 5-ml. volume), and by making microscopic manometric readings, this delicacy of measurement may be increased some fifty to one hundred times.

It has been estimated by Dickens and Simer (*19*) and Dixon (*20*) that the total errors inherent in manometric methods of this type usually amount to approximately 2 per cent. The determination of the vessel constant is probably the most accurate part of the technique. With the standard style of Barcroft differential manometer it is possible to determine the apparatus constant, k, according to Equation 2 with an accuracy of at least 0.5 per cent. When a vessel constant becomes as large as 10, the accuracy with which the reading may be interpreted becomes only 0.1 as great as when the constant is 1, and each graduation will represent an internal change of 10 and 1 cu. mm., respectively.

Singh and Mathur (*76*), using a Barcroft respirometer, found the accuracy of the apparatus to be ± 1.25 per cent, when used for quantitative determinations of known amounts of carbon dioxide liberated by adding measured amounts of standard acid from the vessel side arms to an excess of a bicarbonate solution contained in the main part of the vessel. When applied to the determination of a specific substance, such as carbon monoxide in blood, the Barcroft-Warburg apparatus compares favorably with the well-known referee method of Van Slyke and Neill (*89*). The average discrepancy between these two methods according to Roughton (*66*) was found to be 0.06 volume per cent carbon monoxide (= 0.3 per cent COHb), and in over thirty replicates by the Barcroft method the average discrepancy was 0.12 volume per cent carbon monoxide (= 0.6 per cent COHb).

Johnston and Frey (*43*) found that by using the same reaction vessel for each measurement and carefully controlling conditions, a precision of 1 per cent could be easily attained with the Barcroft-Warburg equipment in measuring the autoxidation induction periods of fatty oils. This study presents an interesting feature in that the determinations were made at 100° C., thus indicating that these methods can be extended to elevated temperatures with convenience and relatively good precision. Corbet and Wooldridge (*18*) investigated the accuracy of the Barcroft differential manometer in respiration studies and concluded that single or even duplicate experiments should be accepted with caution. The experiments should be replicated sufficiently and continued for long enough periods to give a standard error of 5 per cent. The direct method of Warburg in metabolism experiments has been criticized by Brock, Druckrey, and Richter (*13*) on the grounds that when intensive respiration is to be expected (as in the oxygen consumption of eggs) the manometer drops slightly or rises but very little, thus indicating the formation of some compensating gas. The source of error was found to be carbon dioxide formed from sodium bicarbonate by fixed acids, the absorption of carbon dioxide not being instantaneous as is generally assumed.

In summarizing the ultimate factors affecting the accuracy, sensitivity, and speed of response of the Barcroft-Warburg apparatus may be mentioned the limitations arising from:

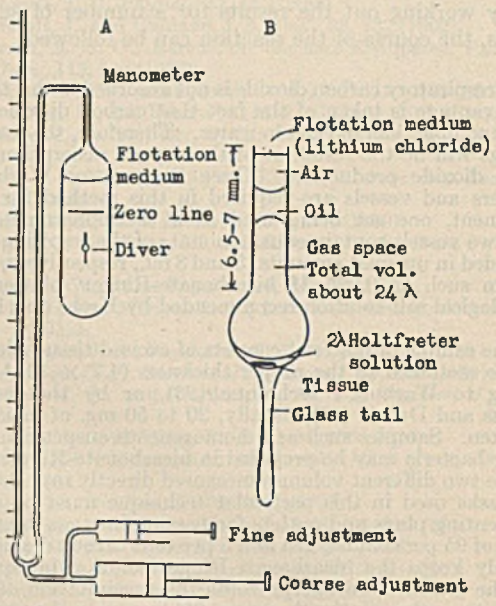

FIGURE 8. CARTESIAN DIVER (B)

Inability of the oxygen to diffuse with sufficient rapidity from the gas into the liquid phase.

Speed of shaking—rate of uptake must be independent of rate of shaking.

Insufficient rate of absorption of carbon dioxide by the alkali present (see work of Dixon and Elliott, *22*).

Use of flasks of improper design and shape, thereby inhibiting free diffusion of gas into liquid.

Diffusion of gases through ground-glass joints.

Practically all the methods employing the Barcroft-Warburg apparatus are based upon the assumption that oxygen and carbon dioxide are the only gases produced or absorbed. Such a statement may be accepted as correct except in the case of certain bacteria, which may evolve hydrogen and other gases; in these instances the methods may be of little value. The presence of excretory nitrogen has also been shown to exert a marked effect on the measurements of the respiratory quotient of single cells or small groups of cells (*80*).

Methods of Measuring Respiration

The present manometric methods for measuring oxygen consumption may be classified into two groups—namely, those in which the oxygen uptake is measured directly, and those in which it is obtained by indirect calculation. (A detailed account of the methods developed by Warburg will be found in the excellent review by Krebs, *48*.) The direct method (*14*, *17*) is, perhaps, the most widely used because of its simplicity and suitability for following the course of the oxygen absorption of a substance.

It is assumed that oxygen is consumed and carbon dioxide is produced during the course of the experiment. The presence of alkali in the inset (central well) of the respiration vessel (see Figure 1) keeps the carbon dioxide tension at zero. A decrease in pressure in the vessel is converted into an equivalent volume assumed to be that of the oxygen consumed. The comparison of pressure values obtained in the manner indicated above and those obtained similarly from a vessel in which no alkali is present gives, presumably, the increase in pressure due to the liberation of carbon dioxide, and these values are in turn converted into the equivalent volumes of carbon dioxide produced.

The indirect method (*1*, *92*, *94*, *96*), on the other hand, indicates the total amount of respiration during a definite period, and enables the oxygen to be measured in bicarbonate solutions,

and by working out the results for a number of successive periods, the course of the reaction can be followed.

The respiratory carbon dioxide is not absorbed in this technique but advantage is taken of the fact that carbon dioxide is more readily soluble than oxygen in water. Therefore, the manometer readings will be the resultant of the oxygen absorption and the carbon dioxide production. Three Barcroft or Warburg manometers and vessels are required in this method for a single experiment, one set being used as a thermobarometer. The other two vessels contain equal amounts of the respiring material suspended in unequal amounts, 3 and 8 ml., respectively, of a fluid medium such as 0.025 *M* bicarbonate-Ringer solution or the physiological salt solution recommended by Krebs and Henseleit (*49*).

If the sample of material consists of excised tissue slices, these may be sectioned to the proper thickness (4.7×10^{-2} cm.) according to Warburg's technique (*93*), or by the method of Thomas and DeEds (*85*), usually, 20 to 50 mg. of moist weight are taken. Samples such as a homogeneous suspension of yeast cells or bacteria may be prepared in bicarbonate-Ringer solution and the two different volumes measured directly into the vessels. The flasks used in this particular technique must be equipped with venting plugs and outlets for passing in a gas mixture consisting of 95 per cent oxygen and 5 per cent carbon dioxide. This not only keeps the bicarbonate-Ringer solution in equilibrium with the gas mixture but prevents the precipitation of calcium carbonate as the solution becomes alkaline upon exposure to the air.

If h and H are the respective readings for the same time interval, and assuming that the respiration rate of the sample is the same in both manometers, the following relationship may be expressed, where k and K are the respective vessel constants:

$$\text{Oxygen uptake} = \frac{hk_{CO_2} - HK_{CO_2}}{\frac{k_{CO_2}}{k_{O_2}} - \frac{K_{CO_2}}{K_{O_2}}}$$

$$CO_2 \text{ evolution} = \frac{hk_{O_2} - HK_{O_2}}{\frac{k_{O_2}}{k_{CO_2}} - \frac{K_{O_2}}{K_{CO_2}}}$$

By the application of the same principle, carbon dioxide assimilation as compared with respiration, fermentation as compared with respiration, and butyric acid fermentation as compared with lactic acid fermentation can be measured manometrically. The indirect method has been of considerable value in the study of animal tissue respiration under physiological conditions, especially in the investigation of tumors. It has been improved upon by Dickens and Simer (*19*) and more directly by Dixon and Keilin (*23*).

A brief outline of the various steps involved in making concurrent measurements of the oxygen consumption and carbon dioxide production according to the direct method may be given as follows:

1. Determine experimentally the time periods required for temperature adjustment. (This can be done by placing the vessels in the bath under experimental conditions and observing the time necessary to bring the manometric fluid to a constant level with the stopcock of the manometer closed.)
2. Place in the side arm of the flask a definite amount of a dilute acid, usually 0.5 ml. of *N* hydrochloric acid, and 0.2 ml. of 30 per cent potassium hydroxide in the adjacent side arm or the central well (inset) of the flask. (This step of the procedure can be modified to meet the individual requirements of the material being studied and will vary with the type of vessel used.)
3. Assemble the manometer and flask and attach to the shaking mechanism, thus submerging the flask in the thermostat. Shake for about 10 minutes to allow the system to become equilibrated at the desired temperature.
4. Remove the unit from the shaker, detach flask, and insert sample of respiring material into main compartment of vessel. Then reassemble the unit and return it to the shaker. Record time *A* as soon as the sample is placed in the vessel and regard this as the beginning of the period of carbon dioxide production.
5. Shake for 3 minutes to allow respirometer to reach temperature of bath, then adjust indicating fluid to the 150-mm. mark, and note reading *B*, on the open arm of the manometer, together with time *C*. Close stopcock. *C* is regarded as the beginning of the period of oxygen consumption. The apparatus is then allowed to operate for the duration of the experiment.
6. Record time *D* at the termination of the oxygen consumption period and then adjust the manometer fluid to the 150-mm. mark, followed by reading *E*. The length of the oxygen consumption period will then be $D - C$; the amount of oxygen consumed will be represented by $B - E$.
7. Remove manometer from shaker and turn the unit in such a position as to permit the acid in the side arm of the vessel to flow into the alkali, thus liberating the carbon dioxide absorbed. Return manometer to thermostat and shake again for 3 minutes. Adjust indicating fluid, followed by reading *F* and time *G*. *G* marks the end of the period of carbon dioxide production.

In making a series of determinations with the Warburg respirometer, a control unit without respiring material (thermobarometer) must be subjected to the same conditions, reagents, and treatment as the series. This provides a correction for any temperature and external pressure changes during the experiment and for any dissolved carbon dioxide in the reagents.

Consideration must also be given to certain other factors if the above-mentioned methods are to represent the true respiration of the tissue slice or cell suspension in question. As previously stated, the kinetics of gas-liquid reactions can

TABLE II. APPLICATIONS

Material Studied	Function Measured	Apparatus Used	Accuracy	Reference	Date
Amino acids	CO_2 evolved	Warburg	...	(*68*)	1938
Arginase (blood)	CO_2 evolved	Warburg	0.003 mg. urea	(*97*)	1934
Ascorbic acid oxidation	O_2 uptake	Barcroft	...	(*7*)	1936
Bacterial growth	O_2 uptake	Warburg	...	(*36*)	1941
Beer oxidation	O_2 consumed	Warburg	...	(*61*)	1940
Carboxylase	CO_2 evolved	Dixon-Keilin	...	(*55*)	1941
Catecholase	O_2 absorption	Barcroft	±2–3%	(*58*)	1941
Coenzyme I (tissues)	CO_2 evolved	Warburg	10%	(*3*)	1939
Coffee oxidation	O_2 absorbed	Warburg	...	(*42*)	1938
Cozymase	CO_2 evolved	Warburg	±0.03 μg.	(*40*)	1941
Enzyme purification	O_2 uptake	Warburg	...	(*4, 9, 27*)	1935
Fatty acid oxidation	O_2 uptake	Warburg	...	(*8, 59*)	1938
Fatty oils oxidation	O_2 absorption	Warburg	1%	(*43*)	1941
Germicides (evaluation)	O_2 uptake	Warburg	...	(*36*)	1941
Glutathione (tissues)	CO_2 production	Warburg	<6%	(*100*)	1935
Glyoxalase	CO_2 production	Warburg	2%	(*60*)	1934
Gases (microanalysis)	N_2 fixation				
	O_2 absorption	Warburg	...	(*15*)	1932
Grains (cereal)	CO_2/O_2	Warburg	...	(*84*)	1942
Hydrogen transport (mechanism)	O_2 uptake	Warburg	...	(*61*)	1940
Hydrogenations (microcatalytic)	H_2 absorption	Barcroft (mod.)	±2%	(*39*)	1930
Insulin and carbohydrates	R. Q.	Warburg	...	(*38, 81*)	1940
Narcotics (brain tissue)	QO_2 absorption	Barcroft	...	(*63*)	1934
Nitrifying bacteria	O_2 consumption	Warburg	...	(*12*)	1939
Phenolase (plant tissues)	O_2 absorption	Warburg	2.5%	(*67*)	1935
Photosynthesis (algae)	O_2/CO_2	Warburg	...	(*50*)	1940
Sewage effluents	O_2 absorption	Barcroft	...	(*101*)	1936
Sulfonamide compounds (bacterial metabolism)	O_2 consumption	Warburg	...	(*47*)	1940
Soils (CO_2 content)	CO_2 production	Barcroft	...	(*79*)	1932
Tea fermentation	O_2 uptake	Warburg	...	(*64*)	1941
Thiamine (biological materials)	CO_2 production	Warburg	102–105% recovery	(*33*)	1941
		Fermentometer	2%	(*70*)	1942
		Warburg-Summerson	2%	(*44*)	1942
Tubercle bacilli (metabolism)	O_2 consumption	Warburg	...	(*46*)	1939
Unsaturated compounds	H_2 absorption	Warburg	0.5%	(*60*)	1934
Urease activity	CO_2 production	Warburg	...	(*78*)	1941
Volatile substances soluble in water	Ether	Warburg	...	(*45*)	1937
Wheat respiration	O_2 uptake	Fenn	...	(*2*)	1938
Wood sections	O_2 consumption	Fenn	...	(*54*)	1940

be recorded manometrically only if the rates so observed are independent of the speed of shaking of the vessel and of the relative volumes of the liquid and gas phases. Otherwise, the observed rates are dependent upon the speed of diffusion of dissolved gas between the two phases and also on the true speeds of the chemical processes.

Allowance can be made for the effect of diffusion in manometric experiments by assuming the existence of a stationary film of liquid, at the boundary between the liquid and gas phases, diffusion through which determines the rate of exchange of gas between gas and liquid. Roughton (*65*) has developed an excellent method of allowing for the influence of diffusion in manometric measurements by carefully studying the kinetics of carbon dioxide uptake and output by buffer solutions, in the presence of carbonic anhydrase. The method is based upon the stationary film theory of gas-liquid interchange which states that when solution of a gas occurs without any chemical reaction

$$\text{Rate of gas uptake} = \frac{D_i}{\delta} A (C_i - C_L) \qquad (3)$$

where

D_i = diffusion coefficient of dissolved gas
δ = thickness of stationary film
A = area of stationary film in sq. cm.
C_i = concentration of dissolved gas at the outer surface of the stationary film = αP_i
α = solubility coefficient of the gas in the liquid
P_i = pressure of the gas in atmospheres
C_L = average molal concentration of the dissolved gas in the bulk of the liquid phase

Equation 3 is also valid for the liberation of dissolved gas from liquid.

Applications

The applications and general usefulness of the Barcroft-Warburg apparatus as an analytical tool for biological and chemical research can hardly be overemphasized. While the greater majority of workers utilizing this instrument have been interested chiefly in purely biochemical or physiological problems, it nevertheless has been and can be used to considerable advantage by workers in allied fields. Typical examples of the diversified applicability of this equipment will be found in Table II. It is not the purpose of this paper to present a complete bibliography, but rather to provide a list of the more important key references and thereby assist the analyst and potential user to select those methods particularly suited to his problems.

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Microdetermination of Carbon in Steels

Modification of the Standard Carbon-Hydrogen Train

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IN JUNE, 1940, the Metallurgical Section of this laboratory asked the authors to determine low percentages of carbon in small samples of Nichrome for the purpose of establishing whether certain segregations formed in this alloy during service were carbides or nitrides.

Since the amounts of carbon involved were far too small for the macrocombustion method, the authors developed a micromethod by introducing a quartz furnace into the standard carbon-hydrogen train (*2*) just before the Supremax combustion tube (universal filling). A modification of some sort is necessary because combustion in ordinary microanalysis occurs at temperatures far below the minimum of 1100° to 1150° C. advisable in the case of steels. More recently, Smoluchowski (*3*) requested carbon determinations on small samples to corroborate routine analytical results obtained in connection with experiments on the diffusion of carbon in steel. For this work, the authors modified the standard microtrain in another way—by slipping a furnace directly over the first part of a standard quartz combustion tube.

Both modifications eventually gave satisfactory results and promise to be particularly useful when such carbon determinations are made only rarely, so that the setting up of a special train is not warranted. The micromethod of Klinger, Koch, and Blaschczyk (*1*) requires a special train.

Experimental

AUXILIARY COMBUSTION TUBE. The quartz auxiliary combustion tube (Figure 1) was calibrated with a thermocouple in the position of the boat, *G*. Standard microchemical technique was observed throughout except for the following details. Granulated tin served as accelerator. A weight of tin equal to half the weight of sample sufficed, although much more was occasionally added. Before steel samples were run, the train was conditioned by burning an unweighed primer of sugar in the conventional way. The auxiliary combustion tube was heated to 900° C. in 10 minutes and to 1150° C. in 25 minutes. The steel sample usually began to burn with a bright glow 3 or 4 minutes after heating was begun. After 30 minutes of heating time had elapsed, the current was interrupted, and the train was flushed for 20 minutes more. An oxygen flow of 5 cc. per minute was maintained for the entire 50-minute period; during the combustion of the larger samples, the rate at which oxygen was introduced had to be increased in order to maintain this constant flow. Suitable blank corrections were determined from time to time on boats containing tin. These corrections, usually near 40 micrograms of carbon dioxide, were applied to the analytical results, which are given in Table I.

The reliability of the method is shown by the last three results, which were obtained on a standard low-carbon steel. Furthermore, the carbon content of the Nichrome samples

TABLE I. RESULTS WITH AUXILIARY COMBUSTION TUBE
(Small Nichrome samples)

Sample	Sample Weight	Tin	Carbon
	Mg.	*Mg.*	%
318A	4.19	34	0.4
322D	3.84	32	1.3
325G	4.10	37	1.1
326A	4.00	24	2.0
	2.24	44	2.0
	2.14	20	2.1
	3.81	36	2.2
327D	3.95	33	0.7
	3.75	36	0.7
328C	4.40	29	1.8
	2.99	32	1.6
329F	4.49	37	2.5
51690[a]	23.8	24	0.24
	46.0	46	0.26
	20.6	19	0.26

[a] 0.28% C by routine macromethod on this low-carbon steel.

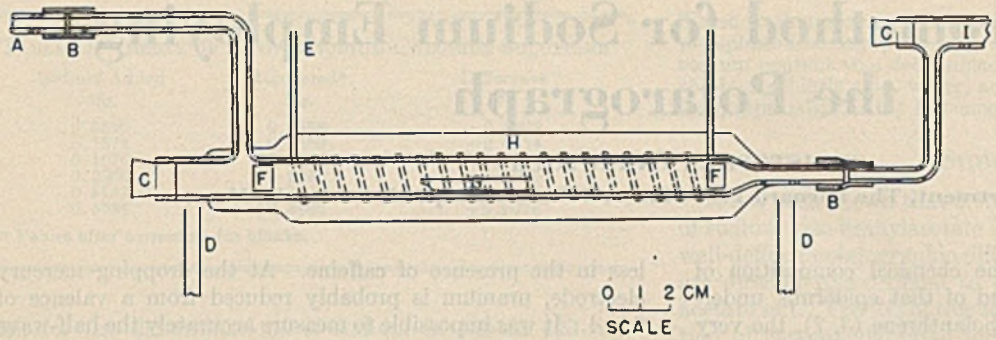

FIGURE 1. CROSS SECTION OF AUXILIARY (QUARTZ) COMBUSTION TUBE

A. Bubble counter
B. Impregnated tubing
C. Corks faced with Al foil
D. Air jets
E. Nichrome tape, 0.025 × 0.16 × 84 cm.
F. Gold foil baffles
G. Porcelain boat
H. Asbestos tape
I. Microcombustion tube

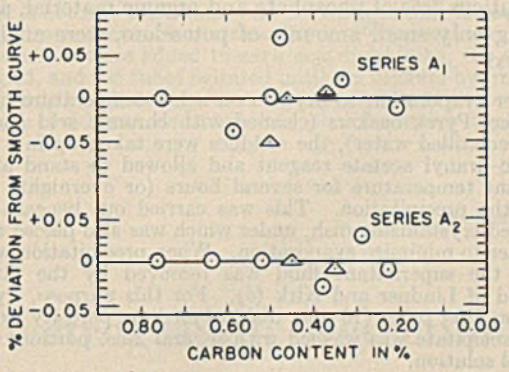

FIGURE 2. PLOT OF GRAPHICALLY OBTAINED DEVIATIONS

△ Micro results
⊙ Macro results

paralleled the amount of the segregated phase shown by the corresponding photomicrographs. This phase could have been only a carbide or a nitride, and nitrogen analyses proved this element to be virtually absent.

When the work was begun, devitrification of quartz and failure of the Nichrome heating units were expected; 20 hours of operation between 900° and 1150° C., however, did not impair the usefulness of the furnace.

SLEEVE FURNACE. In the hope of simplifying the apparatus, a platinum-wound furnace (9 cm. long) was constructed so that it could be slipped over the end of a standard quartz microcombustion tube with 0.5-mm. clearance. During the combustion period, this furnace was placed 0.5 cm. from the entrance end of the long furnace in the microtrain; the sample boat was thus in the position prescribed for the regular carbon and hydrogen microanalysis. A platinum baffle and an air jet were used to protect the cork at the entrance of the combustion tube. With this sleeve furnace, 1150° C. was reached in 5 minutes and maintained for 25 minutes more; otherwise the manipulation was identical with that described above.

The authors soon discovered that the standard quartz combustion tube (walls about 0.8 mm. thick) was too thin for operation at 1150° C. After one failure due to external devitrification, quartz of 1.5-mm. wall thickness was used for the first section of the tube; operation thereafter was satisfactory. The results obtained with the sleeve furnace are given in Table II.

In the analysis of the diffusion specimens, high accuracy was sought. Since the specimens for the microdetermination were taken from between others on which careful macroanalyses had been done, it proved possible to compare the two methods by measuring the deviations from a smooth curve drawn through the carbon contents of all the samples (Figure 2).

The accuracy of the results in Table II can be summarized on the basis of the average difference between the amount of carbon found and the carbon content of the sample calculated from the per cent obtained on the smooth curve referred to above. This average difference for the first ten results in Table II amounts to only 13 micrograms of carbon, in spite of the fact that absolutely uniform samples could not be obtained. If the worst value (0.488 per cent on Sample A1F) is discarded, the average difference for these ten results drops to 7 micrograms of carbon. The average difference between the carbon found and the true value for the Bureau of Standards sample is 3 micrograms of carbon. On the basis of the latter figure, 0.1 per cent carbon in a 100-mg. sample of steel can be determined within ±0.003 per cent.

TABLE II. RESULTS FOR DIFFUSION SPECIMENS OF ORDINARY LOW-CARBON STEEL

Sample	Sample Weight	Tin	Carbon	Average Carbon
	Mg.	*Mg.*	%	%
A1F	17.15	30	0.514	0.501
	106.5	50	0.488	
A1G	25.97	30	0.471	0.465
	100.1	50	0.459	
A1H	103.9	50	0.345	0.354
	105.0	50	0.363	
A2D	105.6	50	0.445	0.449
	102.2	50	0.453	
A2E	104.9	50	0.344	0.347
	49.90	25	0.349	
B. S. 13C[a]	14.12	30	0.539	
	102.9	50	0.574	
	105.3	50	0.571	

[a] Bureau of Standards sample = 0.573% C.

Summary

1. The standard carbon-hydrogen microapparatus can be readily adapted to the microanalysis for carbon in low-carbon steels or Nichromes.

2. In a 100-mg. sample of steel 0.1 per cent carbon can be determined within ±0.003 per cent.

3. The method is particularly useful where only a few samples are to be analyzed at rare intervals.

4. No attempt was made to speed up the determinations.

5. The usual quartz combustion tubes are not suitable for continued use at 1150° C.

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Ultramicromethod for Sodium Employing the Polarograph

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IN INVESTIGATIONS of the chemical composition of isolated mouse epidermis, and of that epidermis undergoing carcinogenesis by methylcholanthrene (*3*, *7*), the very small amounts of tissue available necessitated the adoption of micromethods for the determination of most constituents. This was especially true for sodium. For that metal, a polarographic technique was devised which depends upon the fact that sodium zinc uranyl acetate in dilute hydrochloric acid solution can give well-defined diffusion currents which are directly proportional to the concentration of the triple acetate salt.

Apparatus and Reagents Used

Heyrovský polarograph, model XI (E. H. Sargent & Co.).

Redistilled water for sodium standards, washing, and other operations, prepared in an all-glass (Pyrex) apparatus.

Zinc–uranyl acetate solution, prepared according to the method of Barber and Kolthoff (*1*). This solution was saturated with the triple acetate salt and filtered just before use through a sintered-glass disk covered with a layer of asbestos.

Wash solution, made by saturating 95 per cent alcohol with sodium zinc uranyl acetate, and filtering immediately before using as described above.

Asbestos ("Italian washed and ignited"), ground fine in a mortar, washed on a sintered-glass filter, and suspended in redistilled water.

Experimental

The calibration data (Table I) were obtained from solutions of carefully prepared sodium zinc uranyl acetate in 0.5 *N* hydrochloric acid. The values m and t (where m is the rate of flow of mercury from the capillary, expressed in mg. per second, and t is the drop time in seconds, *4*) were measured in 0.5 *N* hydrochloric acid at a potential of −0.6 volt with respect to the saturated calomel electrode. The diffusion current, i_d, was found proportional to the concentration, C, of sodium zinc uranyl acetate over a range of 0.1625 to 0.975 millimolar, with or without caffeine. This compound easily suppressed occasional maxima. These were, however, of such rare occurrence that the diffusion currents could be measured with or without the alkaloid. The diffusion current and the ratio i_d/C for any particular concentration were significantly less in the presence of caffeine. At the dropping mercury electrode, uranium is probably reduced from a valence of 6 to 4. It was impossible to measure accurately the half-wave potential with the author's instrument, but it was between −0.22 and −0.26 volt with respect to the saturated calomel electrode. Strubl (*6*) found that solutions of uranyl salts in 2 *N* hydroxylamine hydrochloride gave a well-defined wave with a half wave potential of −0.24 volt. The diffusion current was directly proportional to the concentration of the uranyl salts.

Procedure for Solutions

Solutions free of phosphate and organic material, and containing only small amounts of potassium, were analyzed as follows:

After evaporation to dryness on a low-temperature hot plate in 50-cc. Pyrex beakers (cleaned with chromic acid and rinsed with redistilled water), the residues were taken up in 4 to 6 cc. of zinc–uranyl acetate reagent and allowed to stand at nearly constant temperature for several hours (or overnight) to complete the precipitation. This was carried out beneath a large inverted crystallizing dish, under which was also placed a beaker of water to minimize evaporation. When precipitation was complete, the supernatant fluid was removed by the filter-stick method of Lindner and Kirk (*5*). For this purpose, Pyrex immersion filter tubes (10-mm. disk) of medium porosity were used. The precipitate was washed with several 2-cc. portions of wash alcohol solution.

The sodium zinc uranyl acetate in the beaker and on the filter stick asbestos pad was left at room temperature until dry. Then, an accurately measured volume of 0.5 *N* hydrochloric acid was added to dissolve the triple acetate salt. The amount of acid used had to be sufficient to keep the diffusion current in the range of the calibration curve. It was not difficult to estimate this amount of hydrochloric acid after some trials. The solution was passed through filter paper (to remove the asbestos) into small "shell vials", and a trace of caffeine (Merck's anhydrous powder) was added. Shell vials proved to be excellent containers for the electrolysis, since the various sizes available readily accommodated different amounts of solution, and the dropping mercury electrode could be placed well below the surface of the liquid to maintain a good degree of submersion. After removing the oxygen by a stream of nitrogen bubbles, the diffusion current was measured. From this the sodium content could be read directly from the calibration curve.

Table I. Calibration Data for Sodium in Sodium Zinc Uranyl Acetate

Various concentrations of sodium zinc uranyl acetate in 0.5 *N* hydrochloric acid at 25° C. Air removed from solutions with nitrogen. h = 65 cm. Diffusion currents measured at a potential of −0.6 volt with respect to saturated calomel electrode. With caffeine t = 3.385 sec., $m^{2/3}t^{1/6}$ = 2.794 mg.$^{1/2}$ sec.$^{-1/2}$. Without caffeine t = 3.512 sec., $m^{2/3}t^{1/6}$ = 2.904 mg.$^{1/2}$ sec.$^{-1/2}$.

Sodium Zinc Uranyl Acetate		i_d Observed		i_d Corrected		i_d/C	
Millimolar	Sodium equivalent	With caffeine	Without caffeine	With caffeine	Without caffeine	With caffeine	Without caffeine
	Micrograms/cc.	*Microamperes*		*Microamperes*		*Microamp./mmole/l.*	
0.0	...	0.200[a]	0.209[a]	....	....	...	...
0.1625	3.47	1.239	1.348	1.039	1.139	6.39	7.01
0.260	5.98	1.871	.....	1.671	....	6.42	..
0.325	7.47	2.324	2.629	2.124	2.420	6.55	7.44
0.487	11.21	3.384	3.707	3.184	3.497	6.54	7.18
0.650	14.95	4.556	5.026	4.356	4.817	6.69	7.41
0.975	22.43	6.827	7.336	6.627	7.127	6.79	7.31
1.30	29.90	9.302	10.337	9.102	10.128	7.00	7.79
2.60	59.80	19.094	21.270	18.894	21.062	7.26	8.10
3.25	74.80	24.289	27.378	24.089	27.169	7.41	8.36

[a] Residual current of supporting electrolyte alone.

Typical recoveries from pure solutions of sodium chloride are shown in Table II. The accuracy for the amounts of sodium taken was ±0.7 to ±1.5 per cent. Since a 0.1625 millimolar solution of sodium zinc uranyl acetate (equivalent to 3.47 micrograms of sodium per cc.) has a diffusion current greater than one microampere, it is obvious that the method can be used for determining mere traces of sodium.

Procedure for Tissues

In biological materials, interfering substances other than phosphate and organic compounds are rarely found in effective concentrations (*5*). The following procedure has been

Table II. Assay of Known Sodium Chloride Solutions

Sodium Added	Recovered[a]	Difference
Mg.	*Mg.*	*Mg.*
0.0590	0.0598	−0.0008
0.1574	0.1560	−0.0014
0.1970	0.2000	+0.0030
0.2360	0.2380	+0.0020
0.3147	0.3124	−0.0023
0.3934	0.3960	+0.0026

[a] Values after correcting for blanks.

used for the determinations of sodium in 100 to 400 mg. (wet weight) of epidermal tissue.

The samples were ashed at 450° C. (a temperature which has been shown, *5*, to result in no loss of sodium) in a muffle furnace until all the organic matter was destroyed. The ash was then dissolved in 1 cc. of 0.1 *N* hydrochloric acid and this solution washed out of the crucibles into 15-cc. centrifuge tubes with several 2-cc. portions of redistilled water. Phosphate was removed by the method of Butler and Tuthill (*2*) as follows: Calcium hydroxide powder was added to the solutions in the centrifuge tubes, after which they were stoppered and allowed to stand with occasional shaking for 2 hours. The tubes were centrifuged and the supernatant liquid was decanted into clean 50-cc. Pyrex beakers, 2-cc. portions of water were added to each centrifuge tube, the stoppers returned, and the tubes agitated until the calcium hydroxide was resuspended. Centrifugation was repeated, and the supernatant wash fluid was decanted into the respective beakers. Three washings of the calcium hydroxide were carried out. The phosphate-free solutions were evaporated to dryness and the sodium content was determined as described above. Blanks on the hydrochloric acid, water, and calcium hydroxide were necessary, especially for the last-mentioned reagent.

Summary

It has been shown that 0.1625 to 0.975 millimolar solutions of sodium zinc uranylacetate in 0.5 *N* hydrochloric acid give well-defined polarographic diffusion currents. These currents are directly proportional to the concentration of the triple acetate salt. The use of this technique permits the determination of exceedingly small amounts of sodium as in limited samples of biological material.

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Electrographic Detection of Molybdenum in Steel Alloys

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MOLYBDENUM may be detected rapidly by spot tests (*4*, *5*) and tests have been described for detecting molybdenum in steel alloys (*1*). A very rapid electrographic test for molybdenum and molybdenum in steel alloys is described here, which may be completed in a few seconds.

When molybdenum or molybdenum alloy is made the anode in a neutral or slightly acid solution of nitrate ion, using an e. m. f. of 6 to 9 volts and a current of 0.5 to 1.0 ampere, the anode becomes passive (*2*) and the molybdenum and iron enter solution as molybdate and ferric ion, respectively. When a hydrochloric acid solution of stannous chloride and potassium thiocyanate is added, molybdenum forms a complex trivalent molybdenum thiocyanate, yielding a carmine red solution (*4*). Trivalent iron is reduced to divalent by divalent tin. When the test is carried out electrographically, the molybdate is precipitated as lead molybdate with lead acetate solution, which also reduces the concentration of other soluble heavy metal ions, thereby increasing the sensitivity of the test.

Apparatus and Test Solutions

The equipment and procedure are identical with those described in electrographic tests (*2*, *3*).

Test Solution A. Dissolve 30 grams of sodium nitrate, reagent grade, in sufficient distilled water to make 100 ml. and filter.

Test Solution B. Dissolve 1 gram of lead acetate, A. C. S., in sufficient distilled water to make 100 ml. and filter.

Test solution C. Dissolve 30 grams of stannous chloride, A. C. S., in sufficient hydrochloric acid, A. C. S., to make 100 ml. and add to an equal volume of molar potassium thiocyanate. It is advisable to mix only sufficient quantity of the two solutions to last 24 hours.

Method

Fold ashless filter paper to form a wad three to four layers thick and then dip into test solution A. Place the moistened filter paper on the test metal anode and press a graphite cathode about 7.5 cm. (3 inches) long and 0.6 cm. (0.25 inch) in diameter firmly against the free side of the moistened section of the filter paper for about 1 second. An e. m. f. of about 6 to 9 volts is employed and may be obtained by using 4 to 6 No. 6 dry cell batteries connected in series. The current is about 0.5 to 1.0 ampere. A contact of about 3 seconds is recommended for molybdenum steel alloys.

Wash the moistened end of the filter paper with test solution B to remove some of the soluble heavy metal ions and to precipitate the molybdate and fix it to the paper as lead molybdate. Add a few drops of test solution C to the spot on the side of the filter paper that was adjacent to the test metal. The trivalent molybdenum thiocyanate complex formed will yield a carmine red stain.

Molybdenum steel alloys containing 0.2 per cent molybdenum and upwards have been tested. The intensity of the color produced increases with the molybdenum content when the current density and time remain constant. When ferric ion is present, red ferric thiocyanate forms, but ferric iron is reduced quickly to ferrous, which produces a pale green stain that does not interfere with the carmine molybdenum thiocyanate color.

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An Iodoform Microtest for the Higher Alcohols and Ketones

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IN THE study of the lipides of the tubercle bacillus, higher alcohols such as *d*-eicosanol-2 (*1*, *7*) and phthiocerol (*9*) ($C_{36}H_{74}O_3$) have been among the many compounds isolated. To prove the presence or absence of the —CH(OH)CH_3 grouping in these alcohols, it was hoped that the widely used iodoform test might be applied. Unfortunately, the methods heretofore described for carrying out this test were not applicable to these compounds. Scarcity of material or insolubility in water prevented the use of procedures based on the isolation of iodoform for a melting point determination or microscopic study (*2–4*).

These difficulties have now been circumvented by employing the sensitive color test described by Lustgarten (*5*), in which iodoform gives a deep red color when heated with resorcinol and potassium hydroxide. As a solvent for the higher alcohols and ketones, methanol has been found satisfactory. By this method it is possible to detect 1 mg. of eicosanone-2 or 3 mg. of the corresponding alcohol. The fact that the alcohols can be used directly in this test is very helpful in those cases where the amount of material is insufficient to allow oxidation to the ketone. The use of the alcohol is also of advantage in work with compounds such as phthiocerol, which cannot be converted to ketones without disrupting the molecule.

The test was positive with 1-mg. samples of *p*-phenylacetophenone, methyl octadecyl ketone (eicosanone-2), methyl pentadecyl ketone, *o*-benzoxyphenyl acetone, methyl-β-naphthyl ketone, and benzoyl acetone and with 3-mg. samples of *d*-eicosanol-2 and 5,5-dimethyl-2,4-hexanediol. It was negative with citric acid, hydantoin, mannitol, benzophenone, biphenylene oxide, tricarballylic acid, benzyl sulfonamide, and acetyldurene.

Apparatus

The formation of iodoform is carried out in a Pyrex test tube, A, 2.5 cm. in length. This tube is made by fire-polishing the open end (7 mm. in inside diameter) until this dimension is reduced to 4 to 5 mm.

The reaction between the iodoform and resorcinol–potassium hydroxide takes place in a Pyrex test tube, B, 4 cm. long and 7 mm. in inside diameter. A medicine dropper having an opening of 1.5 mm. is used to transfer the contents of tube A to tube B. If the opening is smaller than this, some difficulty may be encountered from solidification of the solution during transfer.

Reagents

SOLUTION I. Three grams of potassium hydroxide are dissolved in 2 cc. of water and 18.7 cc. (15 grams) of pure methanol are added. This solution is stored in a glass-stoppered flask equipped with a medicine dropper.

SOLUTION II. Pure methanol is saturated with iodine with gentle warming. It is kept in the same manner as the alcoholic potassium hydroxide solution.

The Color Test

BLANK TEST. The reagents are tested as follows: Five drops of solution I are pipetted into tube A, and to this solution solution II is added dropwise until a brown color persists. The tube is held for 5 minutes in a water bath at 60° C. and then cooled to room temperature. Sufficient powdered potassium hydroxide is added to discharge the brown color. This is accomplished by rotating the tube on its side, to make sure that the entire inner surface is moistened.

In tube B are placed a few milligrams of resorcinol, which is then covered with a thin layer of powdered potassium hydroxide. This mixture is made slightly moist by the addition of a small drop of water. Tube B is then heated on an asbestos-covered hot plate until bubbles appear. Without removing the tube from the hot plate, several drops of solution from tube A are at once dropped on the resorcinol–potassium hydroxide mixture. One minute of heating should give only a pale green color.

The material under investigation is tested as follows: Because some compounds discolor on warming with alkali or give a red color with heated potassium hydroxide–resorcinol, it is necessary to carry out a blank on 1 mg. of the compound. The material is placed in a small test tube and warmed at 60° for several minutes with 5 drops of solution I. A slight discoloration does not interfere with the test; a deep color shows the compound to be unsuitable for investigation. Several drops of this solution on addition to heated potassium hydroxide–resorcinol should cause no change in the pale green color of the potassium hydroxide–resorcinol. The compounds used in this study met these requirements.

TEST ON KETONES. Five drops of solution I are pipetted into tube A, containing 1 mg. of the ketone to be tested. The tube is held in a water bath until the compound is in solution, and solution II is added dropwise until the brown color appears. The tube is placed in a water bath at 60° for 3 to 4 minutes, solution II being added from time to time to restore the brown color. Sufficient powdered potassium hydroxide is finally added to discharge the brown color when the tube is rotated on its side. If considerable iodoform is present, a yellow color may remain.

In tube B are placed a few milligrams of resorcinol, which is covered by a thin layer of powdered potassium hydroxide. A small drop of water is added to make the mixture slightly moist. Tube B is then placed on an asbestos-covered hot plate and when bubbles begin to appear, several drops of the solution in tube A are added. If the compound tested is a methyl ketone, a deep red color is observed in less than 15 seconds.

TEST ON ALCOHOLS. Eight drops of solution I are pipetted into tube A containing 3 mg. of the alcohol to be tested. The solution is warmed until the compound has dissolved and an excess of solution II is then added. The tube is kept in a water bath at 60° for 3 minutes. By this time, the brown color will have faded, so more of solution II is added to restore it. The solution is heated for another 2 minutes at 60° C. One drop of solution I is added and then an excess of solution II. After another 3 minutes of heating at 60° C., excess powdered potassium hydroxide is added. This solution is added dropwise to moistened potassium hydroxide–resorcinol on the hot plate. If the alcohol is capable of being oxidized to a methyl ketone, a deep red color results in a very short time.

Limitations of the Method

Some halogen compounds [$CCl_3CH(OH)_2$, $CCl_3CHOH.NHCHO$, CCl_3COOH, and $C_6H_2.Cl_3OH$] have been observed by Ware (*11*) to give red colors when heated with resorcinol and potassium hydroxide. In this laboratory $CCl_3.CH(OH)—COOH$ was found to behave similarly; hence care must be taken in the interpretation of results with halogen compounds.

The sterically hindered ketone, acetyldurene, was found to give a negative test. This is to be expected on the basis of the behavior of acetylmesitylene observed by Fuson and Tullock (*4*). For other anomalous haloform reactions, see Suknevich and Chilingaryan (*10*), Slotta and Neisser (*8*), and Paggi (*6*).

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Detection of Zirconium with 5-Chlorobromamine Acid

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BROMAMINE acid is the name used by dye chemists for the sodium salt of 1-amino-4-bromo-2-anthraquinonesulfonic acid. A number of substituted bromamine acids react with the tri- and tetravalent cations in aqueous medium, giving red precipitates. In acid solution, the reactivity is limited to only a few ions, of which the reaction with zirconium is the most sensitive. The chloro derivatives—namely, 5-chloro-, 6-chloro-, and 7-chlorobromamine acid—were found to be the most selective of the substituted bromamine acids studied. Although these three compounds behave similarly, the 5-chloro derivative is recommended because of its slightly more selective reactions. The chlorobromamine acids give heavy bright red precipitates with cadmium, copper, cobalt, nickel, palladium, and zinc in ammoniacal medium.

Although the attempted application of 5-chlorobromamine acid as a quantitative precipitant for zirconium was unsuccessful, the reagent may be used for the detection of zirconium.

Reagents

5-CHLOROBROMAMINE ACID. The compound was obtained from E. I. du Pont de Nemours & Co., Inc. A 50 per cent acetone-water solution containing 2 mg. per ml. of the reagent was used.

ZIRCONIUM. A stock solution containing 1 mg. of zirconium per ml. and 0.2 *M* with respect to nitric acid was prepared from zirconium nitrate and was diluted with water to the desired concentration.

NITRIC ACID. A 2 *M* solution was employed.

Procedure

Transfer 0.05 ml. of the test solution to a depression in a white tile spot plate, and add 0.05 ml. of nitric acid and finally 0.03 ml. of the 5-chlorobromamine acid solution. Shake the spot plate continuously for several minutes before making the observations. A blank prepared in the same manner, except that distilled water is added in place of the test solution, should be used for comparison. Occasionally, the blank may have a small amount of a pale red colored precipitate, which floats on the surface of the liquid and has the appearance of a slight scum. This should not be confused with the precipitate formed in the presence of zirconium, which is more granular and darker colored and settles to the bottom of the solution.

Characteristics of Reaction

The reaction rate is dependent upon agitation of the solution, the concentration of zirconium and of the reagent, and the type and concentration of acid present. Continuous shaking hastens the formation of the precipitate. At the higher concentrations of zirconium the reaction rate is faster than at the lower. The use of a higher concentration of the reagent hastens the precipitation but may cause interference. The reaction rate is about the same in nitric or hydrochloric acid but the latter tends to cause increased interference by the reagent. The reaction rate is greatest at low concentrations of nitric acid and decreases markedly with increasing acid concentration—for instance, using a zirconium concentration of 10 p. p. m. and 0.05 ml. of 0.3 *M* nitric acid, the precipitate forms within a minute; with 2 *M* acid, 2 to 3 minutes are required. The 2 *M* acid is recommended, despite the slower reaction rate, to avoid interference by a number of ions.

TABLE I. LIMITING CONCENTRATION

Ion	Limiting Concentration *Mg./ml.*
Na^+	20
Ba^{++}, Ca^{++}, Sr^{++}, K^+	10
Cd^{++}, Co^{++}, Cu^{++}, Mg^{++}, Mn^{++}, NH_4^+	5
Ni^{++}, Pb^{++}, Zn^{++}	5
Rare earths*, Fe^{+++}, Ga^{+++}, Hg^{++}, In^{+++}, Sc^{+++}, Y^{+++}	1
Ce^{++++}	0.03
Be^{++}	0.25
Al^{+++}, Cr^{+++}, Th^{++++}, Ti^{++++}	0.1
PO_4^{---}, SO_4^{--}	1

* Trivalent ions.

Interference

In testing for the interference of the various ions, the procedure previously given was followed using a zirconium solution containing 10 p. p. m. Solutions of the ions alone, and mixtures of zirconium and the respective ions were used. Suitable blanks were used in all cases. Interference was noted if the ions gave a precipitate in the absence of zirconium and if precipitation was prevented or the character of the precipitate altered in the mixtures of the ions and zirconium. The limiting concentrations given in Table I are for a zirconium concentration of 10 p. p. m. At higher zirconium concentrations they are somewhat greater.

Phosphate and sulfate prevent the formation of the precipitate. Fluoride must be absent. In the case of aluminum, beryllium, chromium, ferric, thorium, and titanium ions, mixtures consisting of zirconium and the respective ions were precipitated as the hydroxides, the precipitates were filtered off and dissolved in nitric acid, and the interference was determined under conditions comparable to the preceding interference studies. The results were comparable to those listed in Table I.

Sensitivity

In the absence of interfering ions, the minimum concentration of zirconium that may be detected is 2 p. p. m. About 5 minutes of continuous shaking are required and a blank must be used. The reaction rate is slow at 5 p. p. m. A zirconium concentration of not less than 10 p. p. m. (0.5 microgram in 0.05 ml.) is recommended.

Colorimetric Determination of Cobalt with Terpyridyl

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IN STUDYING the terpyridyl method for iron (*2*), interference by cobalt was found to result from formation of an orange, complex ion of cobalt and terpyridyl. The constitution of the complex, according to Morgan and Burstall (*1*), is analogous to that of the ferrous complex in which two molecules of the triamine are held by coordinate forces around the octahedral structure of the metal. The two molecules of the base are believed to lie in perpendicular planes determined by the nitrogen atoms.

2,2′-Bipyridyl and 1,10-phenanthroline contain the same iron-specific cyclic N—C—C—N group and both are excellent reagents for iron, but neither gives sufficient color with cobalt to be of interest. 2-Pyridylhydrazine also gives colors with cobalt and iron but is not a satisfactory reagent for either.

The proposed method is simple and direct, requiring only the addition of terpyridyl reagent to a solution of cobaltous ion and measurement of the color which immediately develops.

Experimental Work

SOLUTIONS AND APPARATUS. The color-forming reagent was a 0.1 per cent aqueous solution of terpyridyl (2,6-di-2′-pyridylpyridine) hydrochloride calculated on the basis of the free triamine.

Standard solutions of cobalt nitrate containing 0.5 and 0.05 mg. cobalt per ml. were prepared by dissolving the desired amount of the hexahydrate of reagent grade in redistilled water and making appropriate dilutions.

Adjustments of pH were made with 6 *N* solutions of ammonium hydroxide and hydrochloric acid and buffering was accomplished with small quantities of a 20 per cent ammonium acetate solution.

Standard solutions of the anions studied were prepared from the alkali metal salts in most cases. Nitrates, chlorides, and sulfates were used for the cations. Each solution contained 10 mg. of the ion in question per ml.

Transmittancy measurements were made with a General Electric recording spectrophotometer set for a spectral band width of 10 mμ and pH values were measured with a glass electrode meter.

COLOR REACTION. Three to 5 ml. of the reagent solution should be used for 0.25 mg. of cobalt, or 10 p. p. m. in 25 ml. The slight increase in color intensity produced by doubling the quantity of reagent is not significant. Approximately the same amount of reagent is required for 0.25 mg. of iron. The hue is orange and absorption maxima occur at 445 and 505 mμ. A weak band at about 553 mμ can be isolated with an instrument operating on a spectral band width of 5 mμ or less. The molecular extinction coefficient at 505 mμ is 1360.

EFFECT OF pH. An exceptionally wide variation in pH is permissible, no effect on the intensity or hue resulting from changes between 2 and 10. This range is even wider than that permissible in the determination of iron with terpyridyl.

EFFECT OF COBALT CONCENTRATION. Beer's law is valid for cobalt concentrations between 0.5 and 50 p. p. m., the range most suitable for determinations with a 1-cm. transmission cell. Transmission curves for various concentrations are shown in Figure 1.

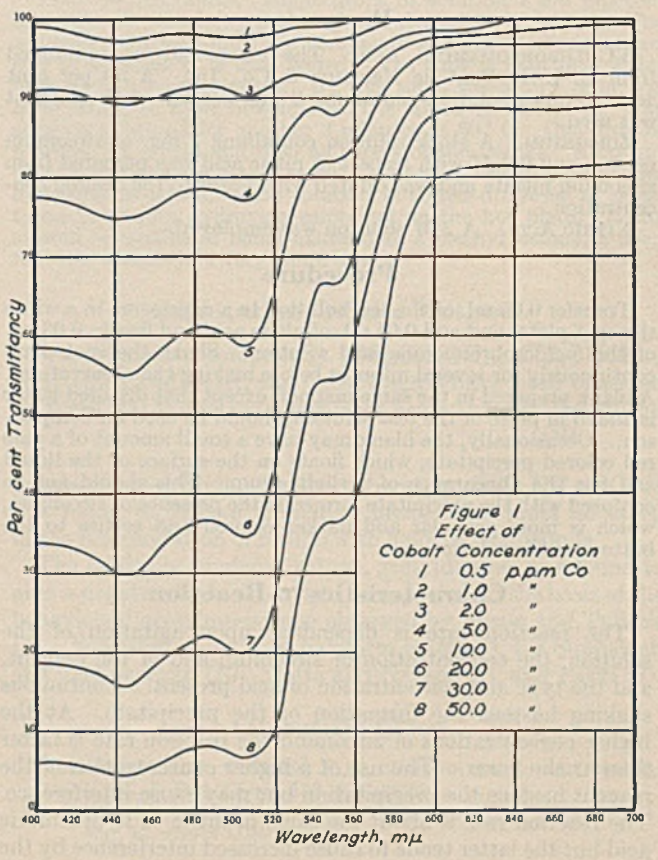
STABILITY OF COLOR. The cobalt complex contains an excess of 1 electron over the number required for maximum stability. Fading occurs within 24 hours at pH 3, necessitating the use of freshly prepared standards if the color measurements are to be made visually. A slight improvement was observed at pH 9, although the cobalt complex appears to be much less stable than the corresponding ferrous complex in any case. Transmission values for the solutions represented in Figure 1 increased 2 to 3 per cent in 24 hours.

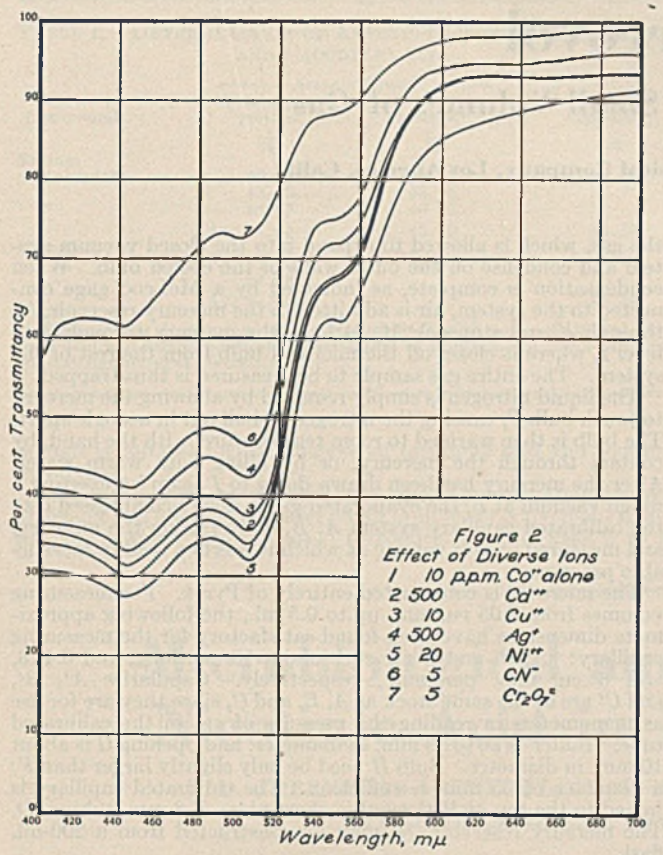
EFFECT OF DIVERSE IONS. In determining the extent of interference by diverse ions, the following procedure was adopted:

The desired volume of solution containing the ion under consideration was added to a 25-ml. volumetric flask containing 0.25 mg. of cobalt measured as cobalt nitrate solution. After addition of 10 ml. of water and 3 ml. of reagent, the solution was diluted to the mark and the transmittancy curve run, using 2-cm. transmission cells with water in the blank cell.

The following ions may be present in concentrations exceeding 500 p. p. m. without causing more than 2 per cent error in the determination of 10 p. p. m. of cobalt: aluminum, ammonium, barium, beryllium, calcium, lead, lithium, magnesium, manganese, potassium, sodium, strontium, thorium, acetate, arsenate, bromide, carbonate, chloride, citrate, fluoride, iodide, nitrate, nitrite, orthophosphate, oxalate, pyrophosphate, silicate, sulfate, sulfite, tetraborate, thiocyanate, and thiosulfate.

The ultimate effect of the interfering ions is summarized in Table I, which shows the approximate limiting concentrations permissible for the determination of 10 p. p. m. of cobalt with an error of not more than 2 per cent. Transmission curves for solutions containing several interfering ions are shown in Figure 2. Iron, cyanide, and dichromate must be absent. Antimony, bismuth, and tin precipitate under the conditions recommended.





Silver interferes slightly and no advantage can be gained by converting it to the ammonia complex. Copper, zinc, and cadmium, if present in sufficiently high concentrations, give low results for cobalt, apparently by complex formation with the reagent.

Titanium, zirconium, and cerium interfere somewhat. Reduction of ceric ion with hydroxylamine reduces substantially the interference of cerium. Uranyl ion, although colored, may be present up to 100 p. p. m.

Of the metals studied, chromium, nickel, copper, and iron cause the greatest difficulty. Attempts to eliminate iron interference by formation of ferric citrate or phosphate were unsuccessful.

Salts of most mineral acids do not affect the color reaction. Certain ions which affect the iron-terpyridyl reaction profoundly, such as carbonate, silicate, and phosphates, do not produce any measurable effect on the cobalt reaction.

Interference by dichromate, cyanide, and vanadate is sufficient to require separations. Five p. p. m. of dichromate caused an error of 70 per cent.

Discussion

Terpyridyl is an excellent reagent for iron according to the characteristics by which colorimetric methods are usually appraised. While the reagent is not so sensitive for cobalt as for iron, it can be used over a considerably wider range of concentrations in the determination of cobalt. The liberal tolerance in control of pH is significant. Most methods employing the organic reagents require considerable care in this respect.

One objection to the proposed method is the instability of the colored complex. Standards for visual comparison should be prepared for each day's work. This is only a minor consideration in view of the increasing use of photoelectric instruments. More serious is the interference of iron, copper, and nickel. Presence of these metals requires separations.

Nitroso R salt, commonly recommended, gives considerably more color with moderate amounts of cobalt (1 to 5 p. p. m.) than does terpyridyl, but for concentrations lower than 0.1 to 0.5 p. p. m. it is of little value because of the yellow color of the reagent itself. The advantage of sensitivity is thus confined to concentrations within the range susceptible to measurement with terpyridyl. Nitroso R salt also requires more care in control of pH and the color appears to be no more stable than that obtained with terpyridyl. Terpyridyl is not available commercially.

Table I. Effect of Diverse Ions

Ion	Added as	Present	Error	Amount Permissible
		P. p. m.	%	*P. p. m.*
Ag^+	$AgNO_3$	100	2	100
Cd^{++}	$Cd(NO_3)_2$	500	4	250
Ce^{++++}	$Ce(NH_4)_4(SO_4)_4$	200	4	100
Cu^{++}	$Cu(NO_3)_2$	5	0	5
Cr^{+++}	$Cr_2(SO_4)_3$	20	3	15
Ni^{++}	$Ni(NO_3)_2$	10	3	5
Ti^{++++}	$Ti(SO_4)_2$	75	3	50
UO_2^{++}	$UO_2(C_2H_3O_2)_2$	100	1	100
Zn^{++}	$Zn(NO_3)_2$	20	2	20
Zr^{++++}	$Zr(NO_3)_4$	100	2	100
AsO_3^{---}(As)	Na_3AsO_3	500	4	300
MoO_4^{--}	$(NH_4)_2MoO_4$	100	5	50
VO_3^-	KVO_3	20	4	10
WO_4^{--}	Na_2WO_4	100	0	100

Recommended Procedure

Treatment of Sample. Dissolve the sample by appropriate means and remove any interfering constituents in accordance with the tolerances listed in Table I. Adjust the pH to 2 to 10. (Twenty per cent ammonium acetate solution, 6 *N* ammonium hydroxide, and 6 *N* hydrochloric acid may be used.) Dilute the solution in a volumetric flask so that each 100 ml. contains 2 to 5 mg. of cobalt.

Measurement of Desired Constituent. Withdraw a 25-ml. aliquot of the sample solution, add 5 ml. of 0.1 per cent terpyridyl solution, dilute to 50 ml., and mix well. (Dissolve the terpyridyl in the least possible amount of 6 *N* hydrochloric acid and dilute to the proper volume with iron-free water.) Measure or compare the color by any of the usual means. (Standards for visual work should be prepared daily.) A blue-green filter such as Corning No. 428 and a 2-cm. transmission cell are recommended for photometric measurement.

Summary

A new colorimetric method for the determination of cobalt is described. Variations in pH between 2 and 10 do not affect the colored complex and Beer's law is valid for cobalt concentrations from 0.5 to 50 p. p. m. This is the optimum range for determinations with a 1-cm. transmission cell. Interference by most of the common metals except copper, nickel, and iron is not serious. Cyanide and dichromate should be absent. Because of the limited stability of the color, fresh standards for visual work should be prepared daily.

Acknowledgment

G. Stafford Whitby of the Chemical Research Laboratory, Teddington, Middlesex, England, very kindly furnished the terpyridyl.

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The Microvol

Apparatus for Measuring Small Volumes of Gas

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A SIMPLE and useful device for measuring very small volumes of condensable gas in a vacuum apparatus is the microvol. Volumes of the order of 0.05 cu. mm. can be measured within 10 per cent, the accuracy improving rapidly with increase in sample size. A single such device can readily be constructed to measure a complete range of volumes from 0.01 cu. mm. up to several milliliters. A microvol has been found very satisfactory in the analysis of the soil atmosphere for substances present in concentrations of less than one part per million, and should be generally applicable in microanalysis of gases.

As shown in Figure 1, the microvol is somewhat similar in construction to a McLeod gage, with the essential added feature of an inner-sealed bulb, *F*, opening to the outside. In use, the microvol is connected to the vacuum system at *E* through a mercury valve or stopcock. The apparatus is thoroughly evacuated, the gas to be measured being contained in a storage tube or held condensed in a trap. Liquid nitrogen is poured into *F* through *G*. The microvol is then opened to the tube containing the gas, which is allowed to expand into the closed vacuum system and condense on the outer walls of the cooled bulb. When condensation is complete, as indicated by a McLeod gage connected to the system, air is admitted to the mercury reservoir, *N*, through *K* and stopcock *M*, to force the mercury through *J* to level *I*, where it closes off the microvol bulb from the rest of the system. The entire gas sample to be measured is thus trapped.

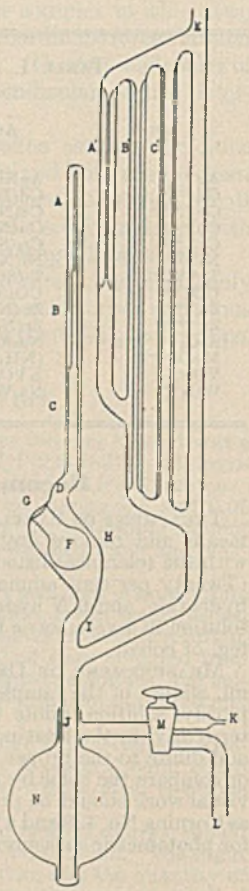

FIGURE 1

The liquid nitrogen is simply removed by allowing the mercury to touch bulb *F*, causing the nitrogen to boil out in a single spurt. The bulb is then warmed to room temperature, with the hand, by contact through the mercury, or by filling with warm water. After the mercury has been drawn down to *I* again by exerting a rough vacuum at *L*, the evaporated gas is slowly compressed into the calibrated capillary system *A*, *B*, *C* by raising the mercury, and measured at any volume at which it exerts a readily measurable pressure.

The microvol is constructed entirely of Pyrex. For measuring volumes from 0.05 cu. mm. up to 0.5 ml., the following approximate dimensions have been found satisfactory for the measuring capillary: *A*, *B*, and *C* are each about 10 cm. long, and 0.4, 3, and 6 cu. mm. per mm., respectively. Capillaries *A'*, *B'*, and *C'* are of the same stock as *A*, *B*, and *C*, since they are for use as manometers in reading the pressure of gas in the calibrated tube. Bulb *F* is 20 to 30 mm. in diameter, and opening *G* is about 10 mm. in diameter. Bulb *H* need be only slightly larger than *F;* a diameter of 35 mm. is sufficient. The calibrated capillary is joined to the top of *H* through a short piece of 8-mm. tubing, *D*. The mercury reservoir, *N*, may be constructed from a 200-ml. flask.

The measuring capillary must be carefully calibrated before sealing to the apparatus. In calibrating the joints, it is convenient to make a permanent mark on the uniform-bore capillary, close to the joint on each side. It is then only necessary to determine the total volume between marks, as the gas volume can be read either above or below each joint with ease. There will be no discontinuities in measurable volumes. Spare capillaries of the same stock can conveniently be calibrated and kept in stock for emergency repairs.

The microvol has the following definite advantages over a McLeod gage used for the same purpose: The entire gas sample, rather than a part only, is directly measured, so that the microvol is more sensitive. The pressure-volume products can be checked very simply by readings at widely different and quickly adjusted mercury levels. A much larger range of volumes can be measured in the same apparatus.

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Iodometric Semimicroprocedure for Determination of Arsenic in Sodium Cacodylate and Cacodylic Acid

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IN THE semimicromethod for the determination of arsenic in organic compounds described in a previous paper (*4*), the samples were decomposed with concentrated sulfuric and nitric acids. However, this method of decomposition is not applicable to cacodylic acid and its sodium salt. Attempts were made to decompose the cacodylate with sulfuric and nitric acids, but when the reduction with hypophosphite was carried out a yellow precipitate formed which volatilized upon heating. Upon further investigation, the most satisfactory method of decomposition for the cacodylate was found to be somewhat similar to the one used for the decomposition of minerals (*2*). This consists of heating the sample with potassium acid sulfate in the presence of small amounts of concentrated sulfuric acid. Specimens of neoarsphenamine were subjected to this treatment without successful decomposition. Therefore the method is recommended for sodium cacodylate and cacodylic acid only.

APPARATUS. The all-glass apparatus recommended for use in the determination of mercury (*3*) and arsenic (*4*) in organic com-

Table I. Determination of Arsenic in Sodium Cacodylate and Cacodylic Acid

Compound	Arsenic Found: Semimicro-method	Arsenic Found: Distillation method[a]	Arsenic Calculated
	%	%	%
Sodium cacodylate	46.63	46.71	
	46.65	46.55	
	46.65	46.85	
	Av. 46.64	46.70	46.83
Cacodylic acid	54.30	...	
	54.38	...	
	Av. 54.34	...	54.31

[a] Sample was decomposed in distillation flask with acid sulfate and sulfuric acid and analysis completed according to procedure of Association of Official Agricultural Chemists (*1*).

pounds is suitable for the analysis of cacodylic acid and sodium cacodylate.

Weigh the dried sample (of size to yield approximately 15 mg. of arsenic) on a piece of cigaret paper or in other convenient manner and transfer to the flask. Introduce into the flask 5 to 6 grams of potassium acid sulfate and 0.5 ml. of concentrated sulfuric acid, attach the condenser, and heat gently until all the potassium acid sulfate is melted. Continue heating vigorously until no further signs of carbon remain, allow to cool, and wash the condenser and walls of the flask with water. Dilute to a volume of approximately 20 ml., insert the glass stopper, and shake until the fused mass is dissolved. Add 3 grams of sodium hypophosphite ($NaH_2PO_2.H_2O$) and follow the original procedure (*4*).

The results in Table I show the method to be satisfactory.

An investigation is being carried out in applying the method to the determination of arsenic in arsenic-containing insecticides, such as calcium, magnesium, lead, and copper arsenates.

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Microbiological and Chemical Assay of Nicotinic Acid in B Complex Products

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THE important role of nicotinic acid (niacin) in human nutrition has stimulated the search for a method of assay which would possess the specificity of the biological assay with dogs and yet afford greater accuracy, speed, and economy. A number of chemical procedures based on the König cyanogen bromide reaction and several microbiological methods have been reported to meet these requirements. The dog method has not been used extensively for quantitative tests, so that comparative data are limited. The recovery experiments and duplication studies in connection with the various rapid methods are subject to limitations as criteria of the reliability of these procedures. It is believed that further comparative assay data from procedures as widely different as the chemical and microbiological tests will provide useful evidence as to their applicability. In the present studies tests have been made on various natural sources of the B complex. The chemical method as modified by Jones (*4*) and the Snell and Wright (*8*) microbiological method were used. Microbiological assays were made by R. D. Greene and A. Black, and chemical assays by F. O. Howland.

Methods

Microbiological. The procedure is essentially that of Snell and Wright (*8*). However, in the preparation of the hydrolyzed casein supplement, the optional charcoal treatment has been used as a regular procedure. In performing this step the authors treat a solution of hydrolyzed casein of pH 3.5, containing about 25 mg. of solids per ml., with 2.5 mg. of Darco G-60 per ml. Recently Isbell (*3*) has reported poor growth with charcoal-treated casein hydrolyzate, due to *p*-aminobenzoic acid deficiency. The authors' hydrolyzate, treated at greater dilution with charcoal, supported optimum growth. Although *p*-aminobenzoic acid was not a limiting factor in their tests, its routine addition seems a desirable safeguard against variations in the casein supplement.

For the preparation of inoculum they have used a dilution of 1 to 25 rather than 1 to 10. For incubation of cultures they have used temperatures of 30° C. and later 37° C. (*9*) with equal success.

In supplying the biotin requirement of the organism they have used the supplement prepared from egg yolk and later pure biotin with equal results. In their experience the free pure biotin at a level of 0.001 microgram per 10 ml. of medium was

Table I. Recovery of Pure Nicotinic Acid in the Presence of Wheat Germ Extract

Added	Found	Recovered	Recovery
Micrograms of nicotinic acid per 10 ml. of medium[a]			%
0	0.096	...	...
0.025	0.122	0.026	104
0.050	0.152	0.056	112
0.075	0.174	0.078	104
0.100	0.203	0.107	107
0.150	0.264	0.168	112
0.200	0.324	0.228	114

[a] Medium according to Snell and Wright +1.4 mg. of wheat germ extract per 10 ml.

Table II. Effect of Acidic Treatment of Samples on Microbiological Assay

Sample No.	Description	Nicotinic Acid: Aqueous	Nicotinic Acid: Acidic[a]
		Mg./g.	*Mg./g.*
2463	Yeast	0.214	0.231
2488	Yeast	0.603	0.599
2514	Yeast	0.218	0.229
2522	Yeast	0.342	0.371
2540	Yeast	0.560	0.488
2560	Yeast	0.506	0.512
2561	Yeast	0.194	0.216
2294	Yeast extract	1.80	1.85
2541	Yeast extract	3.04	2.83
2542	Liver extract	0.508	0.462
2287	Rice bran concentrate	1.64	2.15
2426	Rice bran concentrate	1.18	1.53
2485	Rice bran concentrate	0.97	1.33
2523	Rice bran concentrate	1.02	1.42
2459	Wheat germ extract	0.049	0.069
	Flour, patent	0.0069	0.0095
	Flour, whole wheat	0.0293	0.0362
	Yellow corn, ground	0.0156	0.0194
	Rolled oats	0.0051	0.0057
	Whole milk powder	0.0049	0.0051

[a] Autoclaving for 15 minutes at 15 pounds with 25 volumes of 2 *N* HCl or 2 *N* H_2SO_4.

TABLE III. MICROBIOLOGICAL AND CHEMICAL ASSAYS FOR NICOTINIC ACID

Sample No.	Microbiological[a] No. of tests[b]	Range Mg./g.	Av. Mg./g.	Chemical No. of tests[b]	Range Mg./g.	Av. Mg./g.
Rice Bran Sirup						
15,037	2	1.69–1.74	1.71	1	.....	1.61
13,917	2	1.41–1.42	1.41	1	.....	1.24
12,727	2	1.47–1.47	1.47	1	.....	1.24
9,279	2	1.78–1.79	1.78	2	1.78–1.82	1.80
7,922	2	1.63–1.69	1.66	1	.....	2.10
7,594	2	1.67–1.72	1.69	1	.....	1.50
7,280	2	1.65–1.73	1.69	1	.....	1.90
6,093	2	2.08–2.21	2.14	1	.....	2.20
5,538	2	1.88–1.91	1.89	1	.....	1.80
Dried Yeast						
13,917	2	0.23–0.23	0.23	1	.....	0.36
12,009	6	0.21–0.25	0.23	3	0.17–0.24	0.20
11,322	4	0.34–0.43	0.38	2	0.32–0.35	0.33
10,811	4	0.43–0.46	0.44	2	0.36–0.40	0.38
9,925	2	0.34–0.36	0.35	2	0.37–0.42	0.39
6,532	2	0.54–0.55	0.54	1	.....	0.44
12,874	4	0.52–0.65	0.59	2	0.54–0.76	0.65
11,724	4	0.54–0.62	0.57	4	0.54–0.74	0.60
11,725	2	0.54–0.58	0.56	1	.....	0.39
12,875	4	0.58–0.62	0.60	3	0.42–0.57	0.50
Yeast Extract						
14,236	2	1.63–1.75	1.69	1	.....	2.0
7,633	5	1.79–1.96	1.87	2	1.7–2.5	2.1
6,347	4	1.80–2.22	1.98	2	2.0–2.1	2.05
6,000	2	2.00–2.23	2.12	1	.....	1.8
5,514	2	1.91–1.95	1.93	1	.....	1.8
5,248	2	1.98–2.15	2.06	2	2.1–2.3	2.2
4,871	2	1.91–2.05	1.98	1	.....	1.8
Wheat Germ						
7,991	2	0.067–0.071	0.069	2	0.066–0.071	0.068
7,646	2	0.071–0.071	0.071	2	0.060–0.070	0.065
11,716	2	0.074–0.079	0.076	3	0.064–0.089	0.078
Malted Wheat Germ Extract						
9,414	4	0.096–0.111	0.102	1	.....	0.068
4,902	4	0.087–0.112	0.100	3	0.07–0.13	0.093
98,750	2	0.114–0.115	0.114	3	0.08–0.15	0.123
1,610	2	0.100–0.108	0.104	[a]	.....	...
15,351	2	0.103–0.104	0.103	[a]	.....	...
Cane Molasses						
6A	2	0.0047–0.006	0.0053	1	.....	None
8A	2	0.0098–0.01	0.0099	2	None–0.04	...

[a] All other than yeast products prepared by acid treatment as in Table II.
[b] Independent tests.

more than sufficient for *Lactobacillus arabinosus 17-5*, whereas the methyl ester was almost inactive. Shull, Hutchings, and Peterson (*7*) have reported that the methyl ester is not utilized by *Lactobacillus casei* ϵ.

CHEMICAL. The chemical assays of nicotinic acid were carried out by a modified cyanogen bromide method which has been fully described by Jones (*4*).

Factors Affecting Microbiological Assay

SPECIFICITY OF RESPONSE. In order to test the sufficiency of the basal medium the authors added extracts of rice bran, yeast, and liver in amounts judged by assay to be equivalent to the levels of nicotinic acid in the standard series, up to optimum. The titration curves produced from the addition of these supplements to the basal medium followed the standard curve very closely and indicate that results based on the usual assay range involve negligible stimulation due to substances other than nicotinic acid. This question has been approached in a different way by determining the recovery of varying increments of pure nicotinic acid in the presence of a constant quantity of a wheat germ extract, a product relatively low in nicotinic acid. The recovery data from this experiment, shown in Table I, confirm the results obtained from the addition of the other B complex supplements.

PREPARATION OF SAMPLES FOR ASSAY. Several of the earlier reports on both chemical (*5*, *6*) and microbiological (*8*, *11*) assays of cereals indicated that the results were dependent to a marked degree on how drastic were the conditions of extraction. Recently other reports (*1*, *2*, *10*) have gone into this matter more extensively, showing that cereal products, as distinguished from yeast or animal products, contain a water-soluble precursor of nicotinic acid which yields maximum values in the microbiological assay only after treatment with alkali or strong acid. The question has been raised as to whether this microbiologically inactive precursor of nicotinic acid is active for animals, and the consequent issue as to the available nicotinic acid of cereal foods and feedstuffs must await clarification by further research.

The authors' experiences with a variety of products of cereal origin, ranging from original grains to highly potent concentrates, have paralleled those of other investigators. In order to determine the conditions necessary for the preparation of samples for test, they have made comparative microbiological assays of a variety of products subjected to treatments ranging from aqueous to 4 *N* acid. As a result of these experiments, summarized in Table II, they have adopted the use of strong acid as a standard preparatory treatment for cereals, whereas other materials have been prepared by aqueous extraction.

Microbiological and Chemical Assays

The results of parallel microbiological and chemical assays of various cereal and yeast products are presented in Table III. For the most part the results from the two methods are in agreement and indicate that both these rapid and relatively inexpensive tests are reliable measures of nicotinic acid, although somewhat greater individual variations were observed in the chemical tests of materials of lower nicotinic acid content.

Summary

In agreement with recent reports it has been shown that preliminary acid treatment of cereals, as distinguished from other products, leads to higher microbiological assay values for nicotinic acid.

Comparative assays of various pharmaceutical B complex products for nicotinic acid by the Snell and Wright microbiological method and by the cyanogen bromide method as adapted by Jones have shown substantial agreement between the results from these widely different procedures and tend to establish the validity of both as measures of this factor. In the testing of the materials of lower nicotinic acid content in this series the microbiological method appeared to be somewhat more suitable.

Acknowledgment

In connection with the microbiological data the authors are indebted to Miss Edna Heacock for supervision of stock cultures and to Miss Frieda Bamman and Miss Lois Herbert for technical assistance.

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Spectrochemical Assay for Traces of Tungsten

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A spectrographic procedure which is extremely sensitive in the estimation of traces of tungsten has been developed. The efficiency of the method depends on the tungsten-collecting properties of hydrated alumina when precipitated by sodium bicarbonate in dilute tungsten solution. This collector is efficient in a solution containing any quantity of alkali salts, and with it 0.00002 per cent of tungsten in an ore or concentrate may be isolated and estimated spectrographically.

THE research laboratory of this company was recently faced with the problem of determining trace amounts of tungsten in a series of classifier sands. A spectrographic survey revealed the feasibility of using this instrument in the final evaluation, but a check of the common methods in use for the isolation of tungsten (*3*, *4*) showed these methods to give incomplete separation for trace amounts. An investigation into the possibility of modifying these procedures, or developing others, was thus indicated.

Assay Procedure

Weigh 10 grams of 200-mesh ore or concentrate into a 400-ml. beaker, wet the sample well with about 20 ml. of water, and add 100 ml. of hydrochloric acid. Cover, digest at 60° to 70° C. for about 1 hour, and then boil down to approximately 25 ml. Cool, add 25 ml. of hydrochloric acid and 10 ml. of nitric acid, and again boil to former volume. Add 20-ml. portions of nitric acid with successive boiling down until chemical action is complete. Then add 20-ml. portions of hydrochloric acid until all nitric acid is removed. Take nearly to dryness on a low-temperature hot plate, but do not bake.

Take up in 10 ml. of hydrochloric acid and 20 ml. of water and heat on a low-temperature hot plate until all iron goes into solution. Dilute to 200 ml. and heat to just below boiling. Stir in pulp of about half of a No. 41 Whatman filter paper (12.5-cm.) and continue digestion on plate with occasional stirring for 15 to 20 minutes.

Filter through 12.5-cm. No. 40 Whatman filter paper and wash free of iron with hot 2 per cent hydrochloric acid solution. Both the residue and filtrate are further treated to recover tungsten.

RESIDUE. Ignite gently in a No. 1 porcelain crucible until all organic matter is oxidized, add about 10 grams of powdered fused sodium bisulfate, and mix with residue. Heat in muffle at low temperature until ebullition ceases and then at bright red for about 10 minutes. Cover, remove, and cool until fusion has solidified. Dissolve in about 50 ml. of boiling water and then pour in thin stream into 100 ml. of boiling 10 per cent sodium hydroxide solution, constantly stirred. Boil about 5 minutes, settle, and filter, washing well with hot water. Discard residue.

FILTRATE. Boil down to about 100 ml., adding 10 per cent sodium hydroxide dropwise during the process until ferric hydroxide just fails to persist. Pour this boiling, nearly neutral solution in a thin stream into 150 ml. of vigorously stirred, boiling 20 per cent sodium hydroxide solution in a 1-liter beaker. Cover, place on pad on low-temperature hot plate, and continue boiling about 30 minutes. Remove, add about 500 ml. of boiling water, and allow to settle. Decant and again wash with same quantity of boiling water. Decant, dissolve the residue in the least possible hydrochloric acid, boil down as above, and again pour into boiling sodium hydroxide. Filter decantations through 18.5-cm. No. 1 Whatman filter paper, finally transferring the residue to the filter paper and washing well with hot water. Neutralize the filtrate with hydrochloric acid, evaporate, and combine this filtrate with that from the residue treatment.

To this combined sodium tungstate solution contained in a 1000-ml. beaker, and diluted to 700 to 800 ml., add 3 to 5 ml. of 0.1 per cent methyl orange and after bringing to the neutral point with hydrochloric acid, add 5 to 10 ml. of excess acid. To this solution pipet 10 ml. of aluminum solution, cover, and heat to boiling. Transfer to a medium-temperature hot plate and with constant boiling add saturated sodium bicarbonate solution from a dropping pipet until the hydrated alumina just begins to precipitate. Boil for about 5 minutes, add more methyl orange, and resume the addition of bicarbonate at about 25 to 30 drops per minute until the solution is definitely basic. Add 10 to 15 drops of excess bicarbonate and boil for 2 to 3 minutes. Settle, filter through 12.5-cm. No. 41 Whatman filter paper, and wash with hot water.

Transfer paper and residue to a porcelain crucible, ash in muffle at low temperature and then at 800° C. for 30 to 60 minutes, cool, and weigh the oxides. By means of this weighing, any alumina extracted from the sample, over and above that added, is accounted for. If the increase in alumina is greater than a few per cent, increase the observed value of the tungsten concentration by the same amount to obtain the correct value.

Crush the oxides, but do not grind finely, and weigh 30 mg. into a drilled electrode. Arc together with standards and estimate the intensity of the tungsten line 2896.45 Å.

TABLE I. RECOVERY OF TUNGSTEN

Sample			Recovery		
SiO_2	$FeCl_3$	Tungsten	Residue	Filtrate	Total
Grams	*Grams*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1.0	..	0.20	0.06	0.14	0.20
2.5	..	0.20	0.08	0.11	0.19
..	5.0	0.20	...	0.20	0.20
..	10.0	0.20	...	0.195	0.195
1.0	10.0	0.20	0.065	0.13	0.195
2.5	5.0	0.20	0.075	0.125	0.20

Equipment

A Hilger automatic Littrow-type large quartz spectrograph is used. The arc image is focused upon the slit by means of a Hilger spherical quartz condenser and the exposure is regulated by means of a rotating sector between the lens and slit. The arc source is a motor generator set regulated to deliver 15 to 17 amperes at about 150 volts across the arc and ballast resistance.

Eastman No. 33 antihalation plates, 10 × 25 cm. (4 × 10 inches), are used to photograph the spectrum and are subsequently developed for 6 minutes in D11 developer at 65° F. and fixed in F5 fixer for 20 minutes.

The crushed sample is weighed into ordinary spectrographic graphite electrodes 2.5 cm. (1 inch) long and 0.78 cm. ($^5/_{16}$ inch) in diameter, and uniformly drilled 0.47 cm. ($^3/_{16}$ inch) deep with a 0.39-cm. ($^5/_{32}$-inch) drill. The samples and standards are arced to give a spectrogram as called for in A. S. T. M. specifications for zinc analysis (*1*). The line densities are read on Bausch & Lomb viewing stands by means of B. & L. magnifiers.

STANDARD TUNGSTEN SOLUTION. Prepare a standard solution by dissolving 0.136 gram of tungstic acid in the least possible quantity of dilute sodium hydroxide solution and diluting to 1000 ml. with water. This solution contains 0.1 mg. of tungsten per milliliter.

ALUMINUM SOLUTION. Chemically pure potassium aluminum sulfate is used to prepare a solution containing 10 grams per liter of aluminum. Weigh 168 grams of the hydrate, add 100 ml. of hydrochloric acid, and dilute to 1 liter. For use in the preparation of standards, check the aluminum content of this solution by following the precipitation procedure with sodium bicarbonate, as previously described, and adjusting if necessary.

Preparation of Standards

The standards arced consist of definite amounts of tungsten in an ignited alumina base. The tungsten content of these standards is expressed as a percentage of their aluminum content; thus the 0.1 per cent standard contains 0.1 mg. of tungsten per 100 mg. of aluminum. The tungsten solution for the standards is measured out as follows:

Pipet 2.0 ml. of standard tungsten solution (0.2 mg. of tungsten) into a 100-ml. volumetric flask and dilute to volume. Pipet 50 ml. of this solution (0.1 mg. of tungsten) into a 1-liter beaker containing 10 ml. of aluminum solution (100 mg. of aluminum). Dilute to 700 to 800 ml. and precipitate the hydrated alumina with sodium bicarbonate, as described above, filter, and ignite to obtain the 0.1 per cent tungsten standard. Then dilute the 50 ml. of tungsten solution remaining in the 100-ml. volumetric flask to volume, again pipet 50 ml. with 100 mg. of aluminum, and repeat the precipitation procedure to obtain the 0.05 per cent standard. Continue this pipetting and diluting of the tungsten solution until the 0.0016 per cent standard is obtained. This was the ultimate dilution discernible by means of the author's spectrograph.

Table II. Recovery of Tungsten

	Residue from Preliminary Acid Treatment of 10-Gram Sample		Filtrate from Preliminary Acid Treatment of 10-Gram Sample		
	(2)	(3)	(4)	(5)	
(1) Weight	Tungsten recovered in first $NaHSO_4$ fusion	Tungsten recovered refusing insoluble from (2)	Tungsten recovered after first iron removal	Tungsten recovered on retreating iron ppt. (4)	Total Tungsten Recovered
Gram	*Mg.*		*Mg.*	*Mg.*	*Mg.*
0.23	0.024	N. D.[a]	0.003	0.001	0.028
0.14	0.023	N. D.	0.004	N. D.	0.027
0.29	0.015	N. D.	0.010	N. D.	0.025
0.17	0.012	N. D.	0.008	0.002	0.022
0.63	0.006	N. D.	0.003	N. D.	0.009
0.47	0.008	N. D.	0.002	N. D.	0.010
0.67	0.006	N. D.	0.004	N. D.	0.010
0.32	0.006	N. D.	0.007	0.001	0.014

[a] None detected.

Development of Procedure

In the preliminary spectrographic investigation, using various carriers, several persistent and satisfactorily graded lines in the tungsten spectrum were observed—namely, at 2656.54, 2896.45, and 2946.98 Å. (*2*). The use of the spectrograph as a means of final evaluation thus appeared possible and it was on the basis of spectrographic estimation that the procedure outlined was developed.

The common methods in use for the determination of tungsten claimed no great completeness in the isolation of traces of the element, so a preliminary check of these procedures became essential.

Using a pure sodium tungstate solution, the procedure of Schoeller and Powell (*4*) was followed up to the ignition of the tungsten-tannin-cinchonine complex. The ash was then dissolved in ammonium hydroxide, the ammonia boiled off, a standard amount of copper as a carrier added, and the solution sulfated and fumed to dryness. The spectrogram of this solid was then compared with that of copper sulfate, to which the same amount of tungsten had been added directly. It was found that from a solution containing 0.20 mg. of tungsten, an average of only 0.03 mg. was recovered, while none was recovered from a solution containing 0.02 mg. This separation was thus useless as a preliminary for spectrographic estimation.

An effort was then made to isolate the tungsten as mercurous tungstate, precipitating this compound from a carbonate solution with an excess of mercurous nitrate as outlined by Hillebrand (*3*). This precipitate was ashed at a low temperature, taken up in ammonia, and arced with copper sulfate as before. Precipitating from tungsten solutions containing 0.20 mg. per liter and a pH range of 3 to 9, recoveries of 0.09 to 0.04 mg. were obtained. Thus the use of mercurous carbonate as a gathering agent, while more efficient than cinchonine and tannin, was still not quantitative.

The use of a suitable collector appeared to be the most promising possibility. Numerous collectors were tried and it was finally found that hydrated alumina, as precipitated from boiling solution with sodium bicarbonate, gave a quantitative collection of tungsten.

An estimate of the efficiency of the alumina collector was first obtained by collecting various small quantities of tungsten in alumina, filtering, and sulfating the residue. Other samples were prepared by precipitating tungsten-free alumina as before, filtering, adding the same quantities of tungsten as had been collected in the previous series, and sulfating the residue. In all cases the corresponding tungsten lines observed were of the same average density. However, because of the low aluminum content of the aluminum sulfate arced (and thus of tungsten, since in the standards the tungsten is a definite percentage of the aluminum), the tungsten lines could not be discerned below the 0.025 per cent tungsten standard. By using an aluminum compound of lower molecular weight, the increased aluminum and tungsten content of the sample arced results in greater sensitivity of the tungsten lines. With the ignited alumina base, as recommended, the sensitivity was increased so that the 0.0016 per cent tungsten standard could be observed.

Because of the possible danger of loss of tungsten as tungsten oxide at high temperatures, a series of tests was run on standards to determine if the alumina base permitted such loss. A quantity of the standard was ignited strongly at 1000° to 1100° C. for 2 hours and this sample compared with the original, which had been ignited at a moderate temperature (<800° C.). In all cases, the standards showed no change in line density.

In the application of this procedure to ore analysis, it was found necessary to extract tungsten from both the residue and filtrate after the initial acid treatment.

In Table I are listed some results obtained, using pure silica and ferric chloride. The combined extraction from both the residue and filtrate under the procedure outlined thus appears quantitative.

The tungsten distribution resulting from the preliminary acid treatment of a series of samples and the sensitiveness of the procedure outlined are shown in Table II. The samples are high in sulfides and contain 1.4 to 6.7 per cent of acid-insoluble matter.

All tungsten is recovered from the residue in one fusion. A small additional recovery is made in some cases on retreating the iron precipitate from the preliminary acid treatment filtrate. Thus two successive extractions are specified in the procedure.

In the procedure outlined for ores, various other elements are collected by the alumina and are readily observed in the spectrogram. Their collection is not necessarily quantitative. They include lead, arsenic, antimony, tin, zinc, bismuth, and silica, among others, and after ignition are weighed together with the alumina as Al_2O_3. Unless these elements form an unduly large percentage of the oxides weighed and affect the carrier characteristics of the alumina, they will not otherwise change the calculated tungsten content.

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A Fractional-Distillation Microapparatus

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ONE of the most important operations in organic chemical work is distillation. Several excellent working apparatus have been designed during the past 30 years for fractionating macroquantities of liquids under normal and reduced pressures, among which are those of Kubierschky (*4*), Friedrichs (*2*), Widmer (*8*), Midgley (*5*), Othmer (*6*), and Jantzen and Tiedcke (*3*). The latter, with its specially constructed receiver unit, is widely used as a standard apparatus for the separation of high molecular fatty acids in the form of their esters.

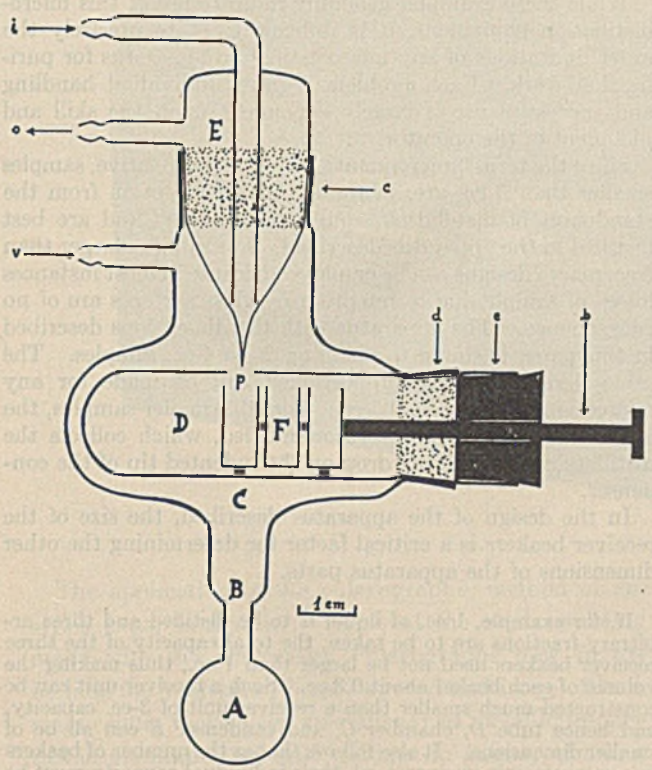

FIGURE 1. DIAGRAM OF APPARATUS

Microdistilling equipment, however, has not received the same concentrated attention, despite the fact that improvements in microchemical procedures generally have kept pace with the tremendous progress in the chemistry of vitamins, hormones, and other biologicals. This may partly be due to the fact that an accurate microthermometer has not been developed. As a result, microdistillations are used primarily to purify samples and not for sharp separation of fractions as in macrodistillations. In fact, much of the dissatisfaction with microdistillations often stems from attempts by operators to make quantitatively sharp fractionations with equipment (including that described herein) which is obviously limited to qualitative purifications. However, arbitrary fractions can be made as a first step to closer refractionation. Some microdistillation apparatus for making arbitrary fractions have been reported, noteworthy among others, those of Craig (*1*) and Shrader and Ritzer (*7*). The limitation of microdistilling equipment to purifications cannot be too strongly emphasized.

Because as much as 5 to 20 per cent of the initial sample in distillations, for all practical purposes, can be considered lost, owing to retention of liquid by the surfaces of the apparatus, it becomes obvious that microdistillation apparatus should be designed with minimum surface areas and minimum distance between the distilling flask and receiver. Only in this way can maximum recovery of sample and distillate be obtained. Unfortunately, some of the published designs do not meet this very important requirement.

In his widely diversified microchemical practice, the author repeatedly encountered need for improved microdistilling apparatus. It was apparent that suitable apparatus, to meet these needs and requirements, could not be built by the simple expedient of reducing the dimensions of efficient macrodistilling apparatus. Consequently a new apparatus was designed in which minimum distance between distilling flask and receiver was obtained by using an "inside receiver" and which also permitted collection of the arbitrary fractions without interruption of distillation.

Because of the size and shape of the new apparatus, exact calculations are either extremely difficult or impossible, and proper proportioning of the parts was made solely on a trial and error basis in various experimental models. Citing the dimension of these earlier models is without value. For most purposes, the dimensions of larger or smaller equipment to purify materials with boiling points falling between 60° and 300° C. can be made proportional to those of the unit here described. If departure must be made from the proportions given, a very elementary principle of distillation should be rigidly adhered to—i. e., the volume of the distilling flask plus the volume of the condenser chamber and connections should be substantially less than the volume which the sample will occupy when transformed into vapor at its boiling point. Unless this principle is observed, reflux action will prevent distillation, especially with high boilers or mixtures with high boiling components.

Apparatus

The apparatus (Figure 1) consists of the distilling flask *A* with a capacity of 3 to 5 ml. The lower section of neck *B* is 5 mm. in diameter; its upper section, 10 mm. in diameter, connects the flask with the chamber, *C*. This chamber has two openings; the one on top is 25 to 30 mm. in diameter and into it is fitted condenser *E* with a ground-glass joint at *c*. (₹ 1/2 ground-glass joints, while not specified for the model which the author is currently using in his practice, will be used in future units.) The condenser is drawn out to a tip and is cooled by a stream of cold water which enters through the glass tube, *i*, filling the condenser and emerging at *o*. Since the effective condenser surface is small, tubes *i* and *o* are of a relatively large diameter (5 mm.) to permit very rapid replacement of cooling water. Tube *D*, 20 to 25 mm. in diameter, lies within *C* and is fitted with a ground-glass joint at *d*. This tube has a 5-mm. hole at *p* through which the distillate drops from the tip of the condenser into the receiver unit, *F*. This unit consists of 3 small beakers of 1-ml. capacity each, made of glass and attached to each other by fusion. It is placed in tube *D* through the opening which is closed during distillation with rubber stopper *e*. *D* has a slightly flattened floor to provide a better footing for the receiver. The two outer beakers of the receiver unit are fitted with glass buttons elevating the beakers 0.6 cm. (0.25 inch) above the flattened floor of *D* to insulate the beakers, so that reheating of the distillate is re-

tarded as much as possible when running high boiling point samples. The receiver is moved under the condenser tip by means of the glass rod, *b*, which passes through the bore of stopper *e*. (*b* is not fused to the receiver unit, as might appear from the picture. Adjustment of the receiver unit could perhaps be made easier at first by providing a glass loop on the unit to be engaged by a hook on the end of *b*.) Since the rod must fit tightly, it may be lubricated with glycerol if necessary. The apparatus is easily and quickly assembled and cleaned.

Operation is very simple. The liquid to be distilled is placed in *A* through the top opening by means of a pipet. For vacuum distillations, a porcelain chip is added to suppress bumping. However, even if bumping occurs, the collected distillate is not likely to be contaminated, since the receiver is almost entirely shielded from splashed or entrained liquid. This protective arrangement, which also prevents the rubber stopper from coming into close contact with the vapors, is one of the principal features of the design. Heat is applied according to requirements, by direct flame, a constant-temperature bath, or electric hot plate.

For best results heat should be slowly and carefully applied. All the distillations cited, including the acetone–ethyl alcohol mixtures, required a minimum of 15 minutes. Since microdistillations are usually run without temperature readings, a fair degree of temperature control is obtained by the thermometer of the bath or a calibrated rheostat in the hot-plate line. The apparatus may be used for distillation under normal or reduced pressures. Its efficiency has been tested with many liquids with boiling points from 60° to 300° C. as follows:

Three groups of liquids were selected for tests with boiling points under 120° for distillation at normal pressure, 120° to 200° for distillation at about 15 mm. pressure, and 200° to 300° for distillation at about 0.1 mm. For the first group, 3-cc. samples of acetone and ethyl alcohol mixtures were made in the ratios of 1 to 1, 1 to 2, and 1 to 3. On distillation of the 1 to 1 mixture, the first fraction of about 1 cc. was found to be pure acetone, as evidenced by refractive index and odor. The third fraction, also of about 1 cc., was found to be pure ethyl alcohol. Similar good results were obtained for the other ratios.

For the second group a mixture of benzaldehyde (boiling point 179°) and benzoyl chloride (boiling point 198°) was distilled at a pressure of about 15 mm. Carbon and hydrogen determinations showed the first and third fractions to be practically pure separations.

For the third group a mixture of lauric acid (boiling point 225°) and myristic acid (boiling point 250°) was distilled at 0.1 mm. and gave almost complete separation of these two fatty acids. Separate refractionation of the first and third fractions then yielded pure fatty acids as determined by elementary analyses.

The efficiency of separation of any distillation equipment is governed by many factors. The one factor which imposes greatest limitation on a microdistillation apparatus is that of the range of difference in boiling points of components in a mixture. In the equipment described, mixtures with components having only a 20° difference in boiling points are readily separated without interruption to collect the lower fraction. As the difference increases to about 40° continuous fractionation becomes increasingly difficult, and with a difference of over 50° fractions must be taken off separately. When continuous separation is attempted, the lower boiling component, depending on the vacuum used, will either be exhausted by the vacuum system, or condensed, reboil, and also be discharged by the vacuum system.

Thus for a mixture involving components with more than a 50° difference in boiling points, this apparatus can recover only one component continuously, though both may be recovered if the convenience of continuous operation is sacrificed. For materials with boiling points above 250°, fractionation becomes increasingly difficult because of bumping tendencies, larger losses due to surface wetting, and the tendency for refluxing to occur at higher distillation temperatures.

Microdistillation apparatus is intended primarily for purifications by means of distillation methods. Several practical cases encountered by the author in his practice illustrate its use.

A research residue of about 5 cc. was submitted for purification and confirming identification. The sample was thought to be quinoline (boiling point 238°) containing about 10 per cent of aniline (boiling point 184°). On distilling a 3-cc. sample, the last two beakers of the receiver (about 1 cc. each) contained pure quinoline as determined by elementary analysis. The fraction in the first beaker proved to be aniline.

Another research residue consisting of γ-picoline (boiling point 143°) containing about 20 per cent of α-picoline (boiling point 128°) was separated at 15 mm. and the last two beakers (1 cc. each) yielded pure γ-picoline checked by refractive index. Ten per cent of the 4-cc. sample distilled was lost.

In the above cases both components were recovered and identified. An example of wide differences in boiling points was presented by purification of a glycerol-water mixture submitted for identification of glycerol. Because a vacuum of 0.1 mm. was used, the water estimated to be about 10 per cent could not be collected, since at this pressure it passes into the vacuum system without condensing. Pure glycerol was obtained and checked by both refractive index and elementary analysis. Only 75 per cent of the glycerol was recovered. The comparatively high loss of 25 per cent was caused by heavier wetting films due to high viscosity.

While these examples generally indicate use of this microdistillation equipment, it is difficult to state precisely the exact limitations of any microdistillation apparatus for purification work. Each problem requires individual handling and successful use is largely dependent upon the skill and judgment of the operator.

Since the term "microquantities" is purely relative, samples smaller than 5 cc. are, in the author's conception from the standpoint of distillation, considered "micro" and are best distilled in the apparatus described. For samples larger than 5 cc. macro designs can be employed because in most instances losses of sample due to retention by glass surfaces are of no consequence. The apparatus with the dimensions described in this paper is suited to distilling 2- to 4-cc. samples. The same design properly proportioned can be made for any microquantity down to 0.5 cc. For still smaller samples, the apparatus of Craig (*1*) is recommended, which collects the distillate as an adhering drop on the indented tip of the condenser.

In the design of the apparatus described, the size of the receiver beakers is a critical factor for determining the other dimensions of the apparatus parts.

If, for example, 1 cc. of liquid is to be distilled and three arbitrary fractions are to be taken, the total capacity of the three receiver beakers need not be larger than 1 cc., thus making the volume of each beaker about 0.3 cc. Such a receiver unit can be constructed much smaller than a receiver unit of 3-cc. capacity, and hence tube *D*, chamber *C*, and condenser *E* can all be of smaller dimensions. It also follows that as the number of beakers in a receiver unit are increased, their individual capacity must be decreased in order to maintain their proper relation to tube *D*, location of hole *p*, and the condenser tip.

Since parts can be easily made in proportion, an apparatus of proper size can be made for any microquantity from 0.5 to 5 cc., making it for all practical purposes a truly universal microdistilling apparatus.

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Presented before the Division of Analytical and Micro Chemistry at the 104th Meeting of the American Chemical Society, Buffalo, N. Y.

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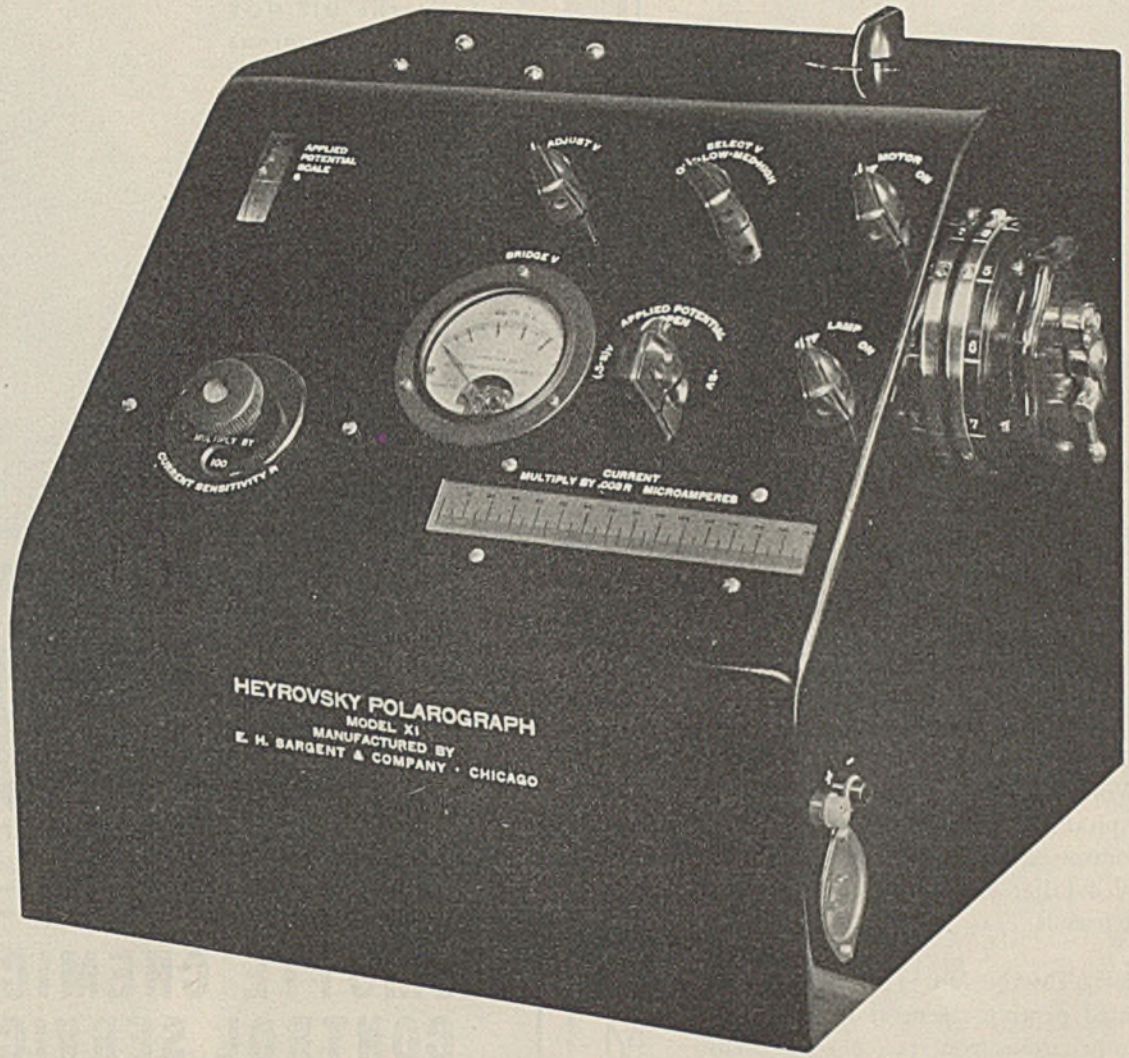

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