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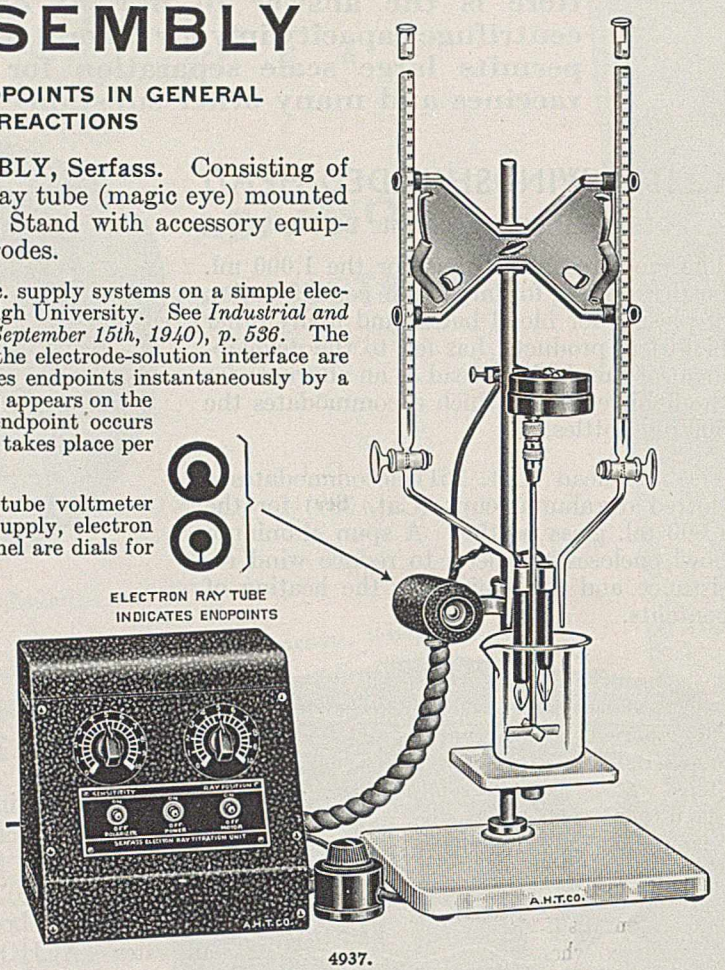

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Determination of Biochemical Oxygen Demand and Dissolved Oxygen of River Mud Suspensions

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IN STUDYING pollution contributed by organic matter deposited on the bottoms of flowing streams, it is frequently necessary to determine dissolved oxygen in suspensions of river muds. Dissolved oxygen in such suspensions has been determined in the past by either the short or Rideal-Stewart modifications of the Winkler method. Stephenson (*2*) has reported recently that the test for dissolved oxygen absorbed (B. O. D. test) is unreliable when applied to turbid unfiltered waters. When the initial dissolved oxygen content of a mud suspension is calculated from the dissolved oxygen of the dilution water (the mud being septic) it has frequently been observed that this calculated initial value is higher than that found by these analytical procedures. The above apparent oxygen loss is similar to the immediate loss of oxygen that is observed when septic sewage, effluent, or stream water is diluted with water of a known dissolved oxygen content. These immediate oxygen losses have been referred to as the immediate or chemical oxygen demand of the material under observation.

The present study of mud suspensions has shown that this so-called immediate oxygen demand is not a true measure of the chemical demand but is largely an apparent oxygen loss due to the failure of the analytical methods employed. Improved analytical procedures are proposed which give a better approximation of the true dissolved oxygen content under the adverse conditions met in the examination of mud suspensions. The time required under aerobic conditions to eliminate the interfering substances has been investigated, the effect on the biochemical oxygen demand of keeping the solids in suspension throughout the incubation period has been studied, and a procedure for determining the true biochemical oxygen demand of these muds is outlined.

Method of Study

The mud samples used in this study, which were septic and viscous, containing from 38 to 68 per cent solids, of which 6.07 to 10.1 per cent were volatile matter, were obtained from various points on the Scioto and Ohio Rivers. One-quarter strength Formula C (*5*) dilution water previously stored for 5 days at 20° C. was used in making all dilutions. The various dilutions were made by introducing the appropriate amount of the sample under the surface of the dilution water, mixing with as little agitation as possible, and siphoning (with continued stirring) into the appropriate bottles. All dissolved oxygen determinations were made in triplicate. The pH determinations were made electrometrically employing the glass electrode, and standard methods (*1*) were followed in all other determinations.

Analytical Data

The dissolved oxygen content found by the Rideal-Stewart modification of the Winkler method in a series of suspensions of one sample of Scioto River mud indicated a so-called immediate chemical demand of about 1000 p. p. m. This value is typical of mud samples from this portion of the river.

In another experiment the mud was suspended in dilution water as described and the dissolved oxygen was determined immediately afterward by four different procedures. The short Winkler and alkaline hypochlorite methods were applied to the untreated suspension and the short Winkler method was also applied to centrifuged and flocculated portions of the sample. The two latter modifications were carried out as follows:

Four centrifuge bottles were completely filled with suspensions, stoppered, and centrifuged at about 2000 r. p. m. for 5 minutes, after which the supernatant was siphoned into three 300-ml. dissolved oxygen bottles and the short Winkler procedure applied. To the suspension filling a 1-liter bottle (capacity of about 1150 ml.) 10 ml. of a 10 per cent alum solution were added, followed by 1 to 2 ml. of concentrated ammonium hydroxide, the stopper was inserted, and the contents were mixed by twirling for about a minute. After allowing the "alum floc" to settle for 10 minutes, the clear supernatant was siphoned into three dissolved oxygen bottles and the short Winkler procedure applied. In removing the suspended solids by either centrifuging or by flocculation, it had been found that there was no atmospheric oxygen pickup during these manipulations even at oxygen concentrations as low as 1.5 p. p. m., providing the usual precautions were taken.

The results obtained in this experiment are shown in Table I. The data indicate that the initial dissolved oxygen results obtained with the short Winkler method were from 8.47 to 74.7 per cent lower than the calculated initial, depending upon the dilution examined. With the alkaline hypochlorite method the results obtained were from 5.84 to 52.8 per cent lower than the calculated initial. When the suspended solids were removed by centrifuging, the initial dissolved oxygen results were only from 1.26 to 15.7 per cent lower than the calculated initial for the different dilutions. Finally, with the flocculated portion the initial results checked with the calculated in the highest dilution and were only from

TABLE I. IMMEDIATE DISSOLVED OXYGEN DATA ON SCIOTO RIVER MUD SUSPENSIONS

Dilution of Mud	Dissolved Oxygen: Calculated initial A	Short Winkler B	Alkaline hypochlorite C	Centrifuged portion D	Flocculated portion E	Immediate Apparent Oxygen Depletion: A − B	A − C	A − D	A − E	Immediate Apparent Oxygen Depletion: $\frac{A-B}{A}\times 100$	$\frac{A-C}{A}\times 100$	$\frac{A-D}{A}\times 100$	$\frac{A-E}{A}\times 100$
	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	%	%	%	%
1/250	7.88	1.99	3.72	6.64	6.77	5.89	4.16	1.24	1.11	74.7	52.8	15.7	14.1
1/500	8.76	6.29	7.04	8.06	8.02	2.47	1.72	0.70	0.74	28.2	19.6	7.99	8.44
1/1000	8.72	6.57	7.51	7.99	8.21	2.15	1.21	0.73	0.51	24.6	13.9	8.37	5.84
1/2000	8.73	7.65	7.77	8.30	8.35	1.08	0.96	0.43	0.38	12.4	11.0	4.75	4.35
1/4000	8.73	7.99	8.22	8.62	8.73	0.74	0.51	0.11	0.00	8.47	5.84	1.26	0.00

TABLE II. STEPS IN ANALYTICAL PROCEDURES

(Interference with dissolved oxygen determination occurs during contact of reagents with river mud suspensions)

Method	Complete Procedure	Preliminary Acid Treatment	Alkalinization and Final Acidification
Rideal-Stewart	1.02	0.88	0.14
Azide modification	1.41	1.14	0.27
		Alkalinization	Final Acidification
Winkler	0.93	0.16	0.77
Short Winkler	0.70	....	0.52[a]
	0.79[b]	0.09[b]	0.89[c]
	...	...	0.92[d]
	...	...	1.03[e]

[a] 0.5-minute acid contact before titration.
[b] 5-minute period of alkalinization.
[c] 2-minute acid contact before titration.
[d] 5-minute acid contact before titration.
[e] 10-minute acid contact before titration.

4.35 to 14.1 per cent lower than the calculated in the other four dilutions.

As the mud suspensions were in contact longer in the centrifuged and flocculated portions of these suspensions than in the portions treated immediately by the short Winkler method, lower dissolved oxygen values would be expected in *D* and *E* than in *A* if the losses were true oxygen demands. From the results obtained it may be inferred that a reaction occurs between the suspension and some or all of the reagents of the Winkler or Rideal-Stewart procedures during the time of contact between the two, thus resulting in lower iodine titers. When the suspended matter was largely removed by centrifuging or flocculation, the larger portion of the interfering materials was removed and the dissolved oxygen results approached the calculated initial values. The experiment indicated that the immediate oxygen losses obtained by the ordinary Winkler procedure, Rideal-Stewart, azide, short, or alkaline hypochlorite modifications are due to interference with the analytical procedures and are not true oxygen demands.

Another series of experiments was performed to determine in which step or steps of the analytical procedures the interference occurred.

For these experiments a dilution of mud was prepared in 4 liters of dilution water. After 10 to 30 minutes of contact with the water, the suspended solids were centrifuged out and resuspended in fresh dilution water. In this way the soluble materials contained in the original mud were largely removed and the effect of the suspended solids only on the dissolved oxygen determination could be followed. In these experiments 10-ml. portions of this suspension containing 101 mg. of suspended solids were added to 300-ml. dissolved oxygen bottles completely filled with portions of the same dilution water at the point in the procedure being studied.

The results of this series of experiments are given in Table II. In the case of the Rideal-Stewart modification it is seen that of the 1.02 p. p. m. loss occurring when the mud was in contact with the dilution water during the whole procedure, 0.88 p. p. m. occurred during the acid-permanganate treatment and 0.14 p. p. m. can be ascribed to the alkalinization and final acidification period. With the azide modification, the greater portion of the loss also occurred during the acid-azide treatment. When the regular Winkler procedure is used 0.77 p. p. m. of the total loss takes place during the final acidification period and 0.16 p. p. m. during the alkalinization period. Finally, in the case of the short Winkler method increasing the period of alkalinization to 5 minutes increased the total loss only 0.09 p. p. m. Approximately 50 per cent of the loss occurring during the final acidification period takes place in the first 0.5 minute.

A comparative study of various methods for determining dissolved oxygen in 1/500 dilutions of river mud showed that alkaline hypochlorite treatment was superior to hypochlorite treatment in an acid medium. A number of variations of the Theriault and McNamee (*4*) alkaline hypochlorite procedure were tried, using various contact periods for the chlorine. If the suspended solids were removed by either centrifuging or flocculation after alkaline hypochlorite treatment, a higher dissolved oxygen content was obtained. This showed that the alkaline hypochlorite treatment was not completely effective in oxidizing interfering substances.

It may be assumed that if the interference was caused by sulfides, these could be removed by treatment with a copper salt. Treatment with varying quantities (2 to 10 ml. per liter) of a 5 per cent copper sulfate solution for 10 minutes before continuing with the Winkler procedure gave dissolved oxygen results that were lower than those obtained following flocculation with alum. A number of coagulating agents were tried, including salts of iron, nickel, copper, lead, and titanium, but none of these showed any advantage over alum in regard to dissolved oxygen recovery, and experiments on their use were not continued.

A condensed summary of the results obtained in this comparative study of procedures is given in Table III. It will be noted that the percentage of recovery of the initial cal-

TABLE III. SUMMARY OF RESULTS OF COMPARATIVE STUDY OF DISSOLVED OXYGEN PROCEDURES

(On 1/500 dilutions of river mud)

Method Studied	Method Rating No.	No. of Comparative Observations (in Triplicate)	Initial Dissolved Oxygen (Calculated)	Dissolved Oxygen Found by Method Studied	Percentage of Calculated Initial Found by Method
			P. p. m.	P. p. m.	
Rideal-Stewart	7	4	8.39	5.57	66.39
Short Winkler	6	6	8.65	5.76	66.59
Alkaline hypochlorite	5	5	8.12	6.95	85.59
Centrifuge	4	8	8.30	7.46	89.87
Alkaline hypochlorite + alum flocculation	3	4	8.55	8.00	93.50
Alum flocculation	2	9	8.29	7.76	93.60
Iodine + alum flocculation	1	4	8.60	8.13	94.50

culated dissolved oxygen ranged from 66.39 for the Rideal-Stewart modification to 94.50 for iodine treatment and flocculation.

This last method consists of adding a slight excess of iodine solution to a slightly acid suspension of the mud, allowing it to react for 3 to 4 minutes, then carefully destroying the excess with a dilute solution of sodium sulfite. The regular alum flocculation procedure is then followed. On the basis of percentage of initial dissolved oxygen recovered, the methods rate in the order shown. On the basis of ease of manipulation and reliability, the plain alum flocculation or centrifuging procedures are to be preferred.

Table IV. Removal of Sulfides and Dissolved Oxygen Determination Interference

(By aeration of 1/250 mud dilution)

	Sulfides			Dissolved Oxygen			
Aeration Time	Standard iodine solution used	Sulfide	Sulfide remaining	Short Winkler method	Alum flocculation method	Interference	Interference remaining
Hours	*Ml./100 ml.*	*P. p. m.*	*%*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*%*
0	1.44	5.23	100	3.13	7.17	4.03	100
0.5	0.80	2.80	53.5	..	..	..	..
1	0.55	2.00	38.2	6.32	8.96	2.64	65.5
2	0.35	1.27	24.3	..	..	..	...
3	0.26	0.94	18.0	..	..	..	...
5	0.15	0.54	10.3	..	..	..	...

Table V. Oxygen Depletions

	Oxygen Depletions			
	Observed Initial D. O.—Observed Final D. O. (*A*)		Calculated Initial D. O.—Observed Final D. O. (*B*)	
Incubation Time	Short Winkler	Alum flocculation method	Short Winkler	Alum flocculation method
Days	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Immediate (apparent initial depletion)	..	..	0.90	0.06
1	1.40	1.58	2.30	1.64
2	2.17	2.72	3.07	2.76
3	2.27	..	3.17	..
4	2.82	3.23	3.72	3.29

Removal of Materials Interfering with Dissolved Oxygen Determination

As the biochemical oxygen demand is dependent upon a final oxygen content after an incubation period, the time intervals required to destroy or oxidize these materials in an aerobic mud dilution should be determined. Several experiments with this purpose in view were performed. Early in this study it was noted that when suspensions of mud were acidified small amounts of hydrogen sulfide were released.

The sulfides were determined by siphoning the suspension under examination into 250-ml. centrifuge bottles until completely filled, adding 2 ml. of concentrated sulfuric acid, stoppering, and centrifuging for 5 minutes. Then 100-ml. portions of supernatant were siphoned off and titrated immediately with a standard 0.025 *N* iodine solution. Using this procedure the sulfides in a 1/250 dilution of mud under aeration for 5 hours were determined at intervals.

The results shown in Table IV indicate that sulfides are removed rather rapidly under these conditions and that only about 10 per cent remain after 5 hours. The interference with the dissolved oxygen determination by the alum flocculation method was also determined on the same sample initially and after 1 hour of aeration, and 65.5 per cent of the initial interference was observed after 1 hour. This and other experiments indicated that the insoluble sulfides in the mud were not the only materials interfering with the dissolved oxygen determination. In another experiment in which the removal of the interference with the dissolved oxygen determination only was followed it was found that 42.7 per cent of the initial interference remained after 5 hours of aeration. In an experiment in which the sample was aerated for 48 hours, 14.5 per cent of the initial interference remained after 24 hours and 10.9 per cent after 48 hours. The indications are that the other interfering materials are removed at a lower rate than the sulfides by aeration and that precautions must be taken in determining the final dissolved oxygen concentration of mud dilutions even after several days' incubation.

Determination of Biochemical Oxygen Demand on River Muds

The difficulties encountered in determining biochemical oxygen demands of river muds may be illustrated by the oxygen depletions calculated from the dissolved oxygen data obtained in an experiment with a 1/2000 dilution. The depletions obtained are shown in Table V.

The ordinary method of determining oxygen depletions as illustrated in *A*, using the short Winkler method, gives fallacious results on mud dilutions for two reasons. First, the 0.90 p. p. m. apparent immediate depletion, which is largely an interference and not a depletion, is not included by the method. Secondly, the final dissolved oxygen observations after incubation may also be low, owing to interference. Consequently, the ordinary procedure for determining depletions gives results which are too low.

With the short Winkler procedure, because of the unreliability of the dissolved oxygen results following incubation, the depletion obtained from the calculated initial dissolved oxygen, shown in *B*, will be too large for the first few days during the incubation period. Consequently, this procedure is also unreliable and cannot be recommended. With the alum flocculation procedure there was little interference in the initial dissolved oxygen determination as indicated by the very small apparent initial depletion, 0.06 p. p. m., in this case. Therefore, the results obtained following the alum flocculation procedure should be more reliable and produce closer approximations to the true oxygen demand, regardless of whether method *A* or *B* is used to determine the depletions. Whether that portion of the demand represented by the immediate oxygen loss, obtained with the use of a corrective dissolved oxygen procedure, would represent a true chemical or biochemical demand is an unanswered question. But in any case the results of this procedure would approximate the total demand for oxygen of the mud under examination and that is the important factor.

Another phase that should be considered in the determination of the oxygen demand of river muds is the effect produced by the settling out of the suspended particles as occurs in the use of the regular method of determining B. O. D. This concentration of the solids in the bottom of the bottles might result in the deoxygenation of the water immediately surrounding the mud particles. This would change the process taking place from one of complete aerobic dissimilation to one in which anaerobic dissimilation took place in the bottom of the bottle and aerobic dissimilation only in the layer above the mud.

With this fact in mind, an experiment was carried out in which the mud particles were kept in suspension by a continuous rotation of the bottles in a specially constructed apparatus kept in the 20° C. incubator and rotating about 1 r. p. m. A duplicate set of samples was also placed in the same incubator and allowed to stand quiescently during the period of the test.

TABLE VI. COMPARISON OF OXYGEN DEPLETIONS

(On 1/250 dilutions of river mud obtained by old procedure and by analysis of clarified portions after continuous rotation during incubation)

	Oxygen Depletions		
	Old procedure, no clarification	Continuous Rotation during Incubation	
		Centrifuge	Alum flocculation
Incubation Period			
Hours	*P. p. m.*	*P. p. m.*	*P. p. m.*
Immediate (apparent initial depletion)	5.35	1.88	1.07
3	6.26	5.22	5.13
4	6.42	5.97	6.14
5	6.70	7.01	6.75
6	7.03	7.64	7.75
7	7.02	8.33	8.39

TABLE VII. COMPARISON OF OBSERVED AND CALCULATED VALUES OF y FOR DILUTIONS OF SCIOTO RIVER MUD

1/250 Dilution at 20° C.			1/2000 Dilution at 20° C.		
Incubation time	Observed	Calculated	Incubation time	Observed	Calculated
Hours			*Days*		
1	2.60[a]	2.06	2	3.50	3.19
2	3.90[a]	3.72	3	4.04	4.23
3	5.13	5.06	4	4.98	5.01
4	6.14	6.13	5	5.46	5.61
5	6.75	7.00	6	5.48	6.05
6	7.75	7.70	7	6.70	6.39
7	8.39	8.26	8	6.52[a]	6.65
..	...	..	9	6.76	6.84
..	...	..	10	7.02	6.99
..	...	..	11	7.19	7.10

[a] Interpolated graphically.

The results of this experiment, as given in Table VI, show how misleading the values might be if the oxygen demand was determined by the usual method alone. From the old procedure one would conclude that the oxygen demand of the mud was 1750 p. p. m. in 7 hours, whereas the alum flocculation method together with continuous rotation shows an oxygen demand of 2100 p. p. m. in the same period.

It is recommended, therefore, that the following steps be observed in the determination of B. O. D. on river mud suspensions: (1) calculation of the initial dissolved oxygen; (2) maintenance of the solids in suspension during the incubation period; and (3) determination of the dissolved oxygen upon the rotated incubated sample after clarification by alum flocculation.

In another experiment a 1/2000 dilution of river mud was rotated and depletions were determined by the alum flocculation procedure, but the period of observation was extended to 11 days. Upon the assumption that this oxidation follows a unimolecular reaction, the Thomas (*6*) slope method of derivation was applied to the data of the last two experiments and the following values for L and k were obtained:

Dilution Used in Experiment	Period of Observation	k (per Day)	L
1/250	3 to 7 hours	2.25	2,650
1/2000	2 to 11 days	0.1214	14,890

When the values of these constants are substituted in the equation $y = L\,(1-10^{-kt})$, and the values of y for each observation time are calculated, it is found, as shown in Table VII, that the observed and calculated values of y check on the whole remarkably well. From these results it must be concluded that there is a change in the rate of oxidation of the mud from the first few hours (as the reaction velocity is independent of the dilutions) to the period following the first day of incubation. The reaction data for the entire period will not fit the unimolecular formula. The course of the oxidation can be fairly well represented, however, as the sum of two unimolecular reactions as first suggested by Theriault and McNamee (*3*). In fact, the similarity of these results to those of Theriault and McNamee may be taken as confirmation of the results these authors obtained when aerating sludge.

Summary

The apparent oxygen loss from the calculated initial dissolved oxygen, obtained when a dilution of mud is immediately examined by the Winkler method, is due to an interference with the analytical procedure.

This interference occurs largely either in the preliminary acid treatment with the Rideal-Stewart or azide procedures, or during the final acidification and titration in the short or regular Winker methods.

Aeration of the diluted mud oxidizes the interfering materials, though 10 per cent of the initial interference may remain after 48 hours of this treatment.

Removal of the solids from the sample reduced the interfering materials and the initial apparent dissolved oxygen loss.

Solids may be removed by centrifuging the sample in completely filled glass-stoppered bottles or flocculating with alum without interfering with the dissolved oxygen content. The alum flocculation procedure gave the better results when applied to river muds.

The B. O. D. determined by the regular dilution procedure as applied to water and sewage is not applicable to river muds because of the interference noted.

A procedure for more accurate determination of the biochemical oxygen demand on river mud dilutions has been proposed.

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PRESENTED before the Division of Water, Sewage, and Sanitation Chemistry at the 99th Meeting of the American Chemical Society, Cincinnati, Ohio.

Correspondence—New Development in Thermionic Relays

In the article on "A New Development in Thermionic Relays" by Waddle and Saeman [IND. ENG. CHEM., Anal. Ed., 12, 225 (1940)], the following statement is made: "If there is danger of grounding the circuit through the control leads, P_1 or P_2, another resistor, R_4, may be inserted to avoid this danger."

The foregoing is, in my opinion, a dangerous understatement of facts. It leaves the installation of the resistor, R_4, to the option of the user of the device and fails to mention that this resistor has any value as regards personal safety.

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In view of Mr. Nutting's suggestions, it would be well to point out the fact that, in the absence of resistor R_4, as indicated on the circuit diagram, severe shocks may be received by the operator if he happens to ground the control circuit through his body.

WALTER SAEMAN

FLORENCE, ALA.

Colorimetric Analysis of a Two-Component Color System

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COLORIMETRY is assuming an ever-increasingly important role in analytical chemistry, and recent improvements in colorimeters and colorimetric methods have increased both the accuracy and speed of analysis. Photoelectric instruments (*8*) designed for use with a series of color filters have largely replaced the visual white light colorimeters in present-day practice. Such instruments are frequently referred to in the German literature as step-photometers or absolute colorimeters. Ashley (*1*) has chosen to call the practice with such instruments "abridged spectrophotometry" and thereby infers the name "abridged spectrophotometer" for these instruments. It is, perhaps, unfortunate and misleading that many of the instruments are referred to by still other names (*2*) or simply as photoelectric colorimeters (*6–9, 13, 14*).

The purpose of this paper is to show theoretically and experimentally how photoelectric filter photometers can be used to resolve the intensity of one color in the presence of a second color which may be present in the solution owing to an interfering ion or substance.

The treatment presented here for a two-component system can be extended to a three-component system or to even more complex systems. Except in unusual cases, however, the resolution of these multicomponent systems in a filter photometer cannot be performed with a great degree of accuracy. Weigert (*12*), using the nearly monochromatic radiation available with a spectrophotometer, reports the resolution of a four-component color system of dyes by a method involving the solution of simultaneous equations in which the extinction coefficients of each color occur.

Theoretical

For the present purpose, it will be convenient to classify all two-component color systems into two groups, those in which spectral separation is possible and those in which spectral separation is not possible in a filter photometer. The first group would include systems where the absorption bands of the two colors do not overlap in the spectral region under examination, while the second group would include those whose absorption bands do overlap in the spectral region under examination.

First, let us consider the case of two colors in solution where there is no overlapping of the absorption bands in the given spectral region. If the analyzing light of a photoelectric photometer be restricted to those wave lengths where only the desired colored substance absorbs light, the presence of the interfering color will not influence the analysis and the system will behave as if only a single color were present. Then, if Beer's law holds,

$$\log \frac{I_0}{I} = kC$$

where I_0 is the intensity of light passing through the pure solvent and I is the intensity of light passing through the colored solution of concentration C. The constant, k, obtained with a given filter depends only on the nature of the absorbing color and the path length of the absorption cell. A straight-line calibration curve is obtained if density, $\log \frac{I_0}{I}$, is plotted against concentration. The slope of this line is numerically equal to the constant, k, in the above equation.

Unfortunately, there are many color systems in which the absorption bands of the components are not sufficiently displaced from each other so that spectral separation can be effected. These systems fall into the second group and require a more specialized treatment for resolution. Figure 1 represents the absorption characteristics of a hypothetical system.

No matter where one selects a filter for component 1, there will be some interference from component 2; however, the maximum in each curve occurs at different wave lengths. By choosing two filters, A and B, whose maximum transmissions correspond to these same wave lengths, a maximum differential in absorption between the two components should be possible. If Beer's law is valid for both components and if each behaves independently of the other in solution, the following theoretical treatment can be applied (*1, 11*). Subscripts 1 and 2 refer to component 1 and component 2, respectively. Superscripts A and B refer to the respective filters. According to Beer's law,

$$\log \left(\frac{I_0}{I}\right)_1^A = k_1^A C_1$$

and

$$d_1^A = k_1^A C_1 \qquad (1)$$

where d_1 is the partial density due to component 1. Similarly, for component 2,

$$d_2^A = k_2^A C_2 \qquad (2)$$

Now the measured density, or total density, D^A, at wave length A is the sum of the partial densities. Thus

$$D^A = d_1^A + d_2^A = k_1^A C_1 + k_2^A C_2 \qquad (3)$$

Similarly, at wave length B we have

$$D^B = d_1^B + d_2^B = k_1^B C_1 + k_2^B C_2 \qquad (4)$$

Solving for C_2 in Equation 3,

$$C_2 = \frac{D^A - k_1^A C_1}{k_2^A}$$

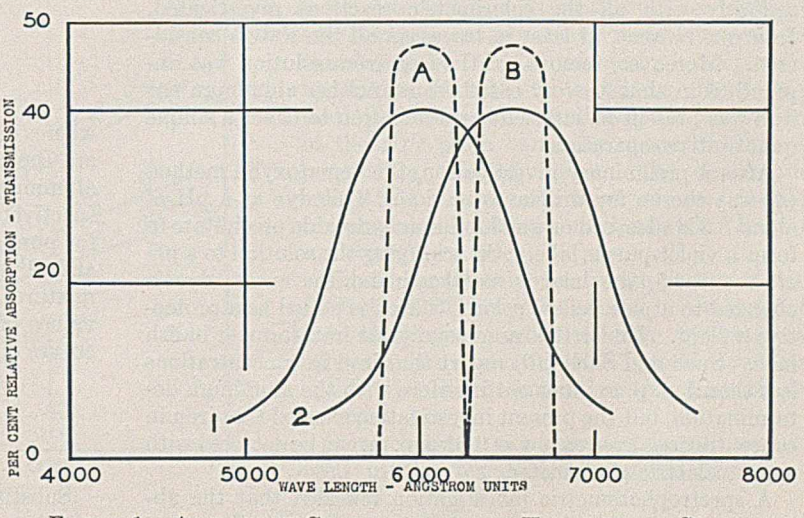

FIGURE 1. ABSORPTION CHARACTERISTICS OF HYPOTHETICAL SYSTEM

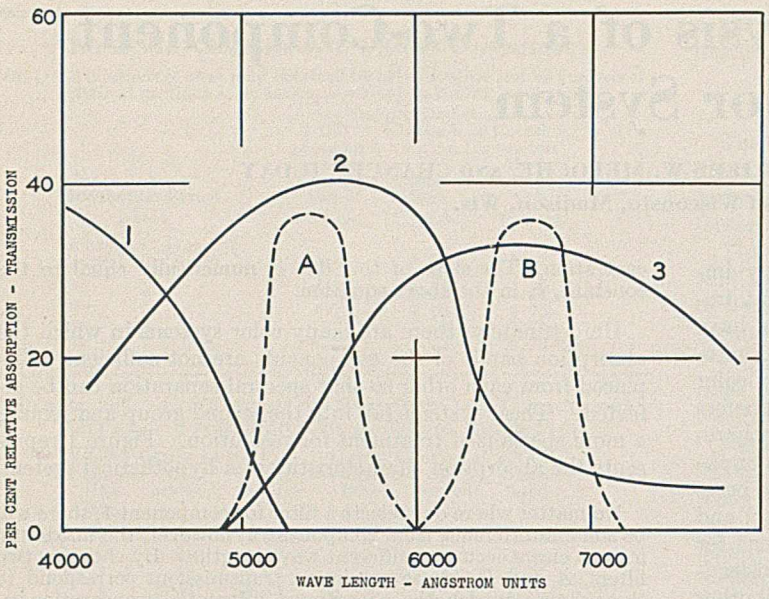

FIGURE 2. ABSORPTION CHARACTERISTICS

1. Absorption curve of hematoxylin dye buffered to pH of 4.5 in concentration used in procedure
2. Absorption of aluminum lake in concentration of 0.5 p. p. m. aluminum
3. Absorption of iron lake in concentration of 0.5 p. p. m. iron
A, *B*. Relative transmission characteristics of filters 540 and 660, respectively

and substituting this in Equation 4, we get

$$C_1 = \frac{k_2^A D^B - k_2^B D^A}{k_2^A k_1^B - k_2^B k_1^A} \qquad (5)$$

By making calibration curves for each of the pure components for each filter, one can obtain the value for the constants in Equation 5 from the slopes of the lines as outlined above.

This makes it possible to solve for the concentration of one or the other component in an unknown mixture of the two by experimentally measuring the densities of the mixture with two appropriate filters.

Experimental

The object of this research was to develop a rapid colorimetric method for the determination of small amounts of aluminum in Northern Wisconsin lake waters. The problem was complicated by the fact that iron interferes rather seriously with all the colorimetric reactions investigated. Iron was present, at least in traces, in all the waters considered. Moreover, removal of the iron from solution was impractical in almost every case because neither aluminum nor iron was present in sufficient concentration to effect a simple quantitative separation.

After a preliminary investigation, the hematoxylin method (*4*) was chosen for this investigation. This dye at a pH of about 8.2 is adsorbed on an aluminum hydroxide precipitate to form a violet-purple lake. On acidifying the solution to a pH of about 4.5, the lake is stabilized and the excess dye is changed to a pale yellow color. Starch is added as a protective colloid. Under the same conditions iron forms a bluish lake. Snell and Snell (*10*) report that iron in concentrations less than 1 p. p. m. does not interfere with the aluminum determination, but the present investigation showed that iron in concentrations even as low as 0.01 p. p. m. can be detected with a photoelectric photometer.

A spectrophotometric investigation revealed that the absorption curves for the two colors overlap each other over most of the spectrum, but that the maximum absorption for each color is in a different region. Figure 2 shows the approximate absorption characteristics of the two colors and the excess yellow dye which also remains in solution after acidification. These curves were plotted from data obtained with an Evelyn photoelectric colorimeter using filters covering eight different regions of the visible spectrum. These data were checked further using a special thermopile-type spectrophotometer (*5*).

Whereas the aluminum and iron colors cannot be spectrally separated, the alternative method of differential spectral separation suggested itself. The filters with maximum transmission at about 5400 Å. for the aluminum color and 6600 Å. for the iron color were chosen for this study. Transmission of light of wave lengths shorter than 5000 Å. is to be avoided because it will cause an error due to the excess dye which absorbs at these short wave lengths.

REAGENTS. Acetic acid, 35 per cent. Dilute glacial acetic acid, 95 per cent, with distilled water.

Ammonium carbonate. Dissolve 50 grams of ammonium carbonate monohydrate in 200 ml. of water. Store in a glass-stoppered bottle in a cool place. Make up fresh every 3 days.

Hematoxylin solution. Dissolve 0.1 gram of C. P. hematoxylin in about 100 ml. of boiling water, cool, and dilute to exactly 200 ml.

Starch solution. Mix 2 grams of soluble starch into a paste and dissolve in 100 ml. of boiling water.

CALIBRATION. The Evelyn photoelectric colorimeter and Evelyn filters Nos. 540 and 660, described above, were used in the calibration and measurements.

Pure samples of iron and aluminum were prepared in concentration intervals of 0.05 p. p. m. from 0 to 0.30 p. p. m. Fifty-milliliter samples were treated as follows: Add 1 ml. of starch solution, 1 ml. of hematoxylin solution, and mix. Add exactly 1 ml. of the ammonium carbonate solution and thoroughly mix the solution. After it has stood 10 minutes, add 1 ml. of 35 per cent acetic acid to produce a buffer mixture which regulates the pH between 4.5 and 4.6. Shake to remove the excess of carbon dioxide formed. Transfer 15 ml. of each sample to an absorption cell and read in the colorimeter immediately, taking a series of readings with each filter. Use distilled water treated in the same way as the reference standard and set at 100 on the colorimeter scale.

Figure 3 shows the density-concentration curves for each component with each filter. The straight line obtained in each case indicates the validity of Beer's law. It was shown earlier that

$$C_1 = \frac{k_2^A D^B - k_2^B D^A}{k_2^A k_1^B - k_2^B k_1^A}$$

where C_1 is the concentration of aluminum; k_1^A and k_1^B are the constants in Equations 1 and 4 for pure samples of aluminum using filters *A* (No. 540) and *B* (No. 660), respectively; k_2^A and k_2^B are the constants in Equations 2 and 4 for pure samples of iron using the respective filters; and D^A and D^B are the total or measured densities of the unknown mixtures of iron and aluminum, using the two filters. The values of the constants are obtained from the slopes of the curves in Figure 3. Thus,

$$k_1^A = 1.895 \quad k_2^A = 0.815$$
$$k_1^B = 0.690 \quad k_2^B = 1.100$$

Substituting these values in the above expression and simplifying, it becomes

$$C_1 = \frac{1.100\,D^A - 0.815\,D^B}{1.511} \qquad (6)$$

Thus, it is possible to solve for the concentration of aluminum in an unknown mixture of iron and aluminum by experimentally measuring the density of the colored solution using two different filters and substituting in the above equation.

PROCEDURE. Concentrate a sample of lake water sufficiently to give a 50-ml. sample containing at least 0.05 p. p. m. of aluminum. Some waters, of course, will not need to be concentrated. Treat this sample as outlined under calibration and measure the density of the colored solution using filters 540 and 660. Substitute the values in Equation 6 and solve for the aluminum concentrations.

RESULTS. Table I shows typical results on eight synthetic samples. Whereas the error in sample 4 appears to be rather high, this determination cannot be taken as typical of the results. The average error for the remaining seven samples is less than ±5 per cent.

TABLE I. TYPICAL RESULTS ON SYNTHETIC SAMPLES

Sample	Galvanometer Readings No. 540	No. 660	D^A	D^B	Supplied Fe	Supplied Al	Al Found
					P. p. m.	*P. p. m.*	*P. p. m.*
1	46.50	68.50	0.332	0.164	0.050	0.150	0.153
2	54.00	65.50	0.267	0.184	0.100	0.100	0.094
3	62.00	63.00	0.207	0.201	0.150	0.050	0.044
4	42.00	49.75	0.377	0.303	0.150	0.150	0.114
5	27.75	53.00	0.557	0.276	0.050	0.250	0.258
6	24.50	57.50	0.611	0.240	0.000	0.300	0.315
7	60.75	63.00	0.216	0.200	0.150	0.050	0.049
8	48.00	56.75	0.319	0.246	0.150	0.100	0.100

Discussion

One of the serious objections to colorimetric analysis in the past has been the difficulty of reading the intensity of the color—i. e., determining the amount of light absorption due to a given component. A second serious objection has been the difficulty of dealing with systems of more than one color—i. e., systems in which an interfering color may occur.

The first objection has been almost entirely eliminated by the introduction of photoelectric devices for measuring light absorption. The second objection is met, at least in part, in this presentation. Although the discussion has been limited to a two-component color system, the theory can be extended to any reasonable number of components. With the recent introduction of a relatively cheap photoelectric diffraction grating spectrophotometer (*3*) into the field of colorimetry, it is not safe to predict the practical experimental limit of the number of color components which can be treated in this way.

The procedure for the development of the aluminum lake as outlined here under calibration differs somewhat from the one recommended by Snell and Snell (*10*). The principal difference is in the concentration of the reagents used. Instead of using 1 ml. of 0.1 per cent hematoxylin solution, the authors recommend 1 ml. of 0.05 per cent reagent, since in low concentrations of aluminum a large excess of dye remains. This is to be avoided because as the concentration of the dye is increased, the absorption of light is extended to the longer wave lengths. This causes an unpredictable error in the measurement of the density with filter 540.

Snell recommends the addition of 1 ml. of a saturated solution of ammonium carbonate to develop the lake. This procedure was found very unsatisfactory, because the stated amount of acetic acid would not reduce the pH to 4.5. Moreover, the solubility of this salt changes rapidly with temperature and, therefore, a saturated solution does not have a definite composition unless the temperature is stated. The authors recommend the use of a 20 per cent by weight solution of ammonium carbonate to be stored in a glass-stoppered bottle in a cool place to avoid as much decomposition as possible. This was found entirely satisfactory when the solution was made up fresh every 2 or 3 days. Using 1 ml. of this carbonate solution to develop the aluminum lake, 1 ml. of 35 per cent acetic acid was just sufficient to reduce the pH to 4.5 to 4.6 as determined with a glass electrode.

The method as outlined is intended for only small concentrations of aluminum in the presence of small amounts of iron. The total concentration of both together should not exceed 0.30 p. p, m. for the calibration given. If the iron concentration is higher than this, a sodium hydroxide digestion and precipitation as recommended by Snell and Snell (*10*) may be found satisfactory. If the total aluminum in unfiltered lake water is desired, a preliminary acid digestion is necessary to make the suspended aluminum available colorimetrically.

This method is not satisfactory for use with highly colored waters containing large amounts of organic materials unless some preliminary treatment is employed to destroy the color and organic materials.

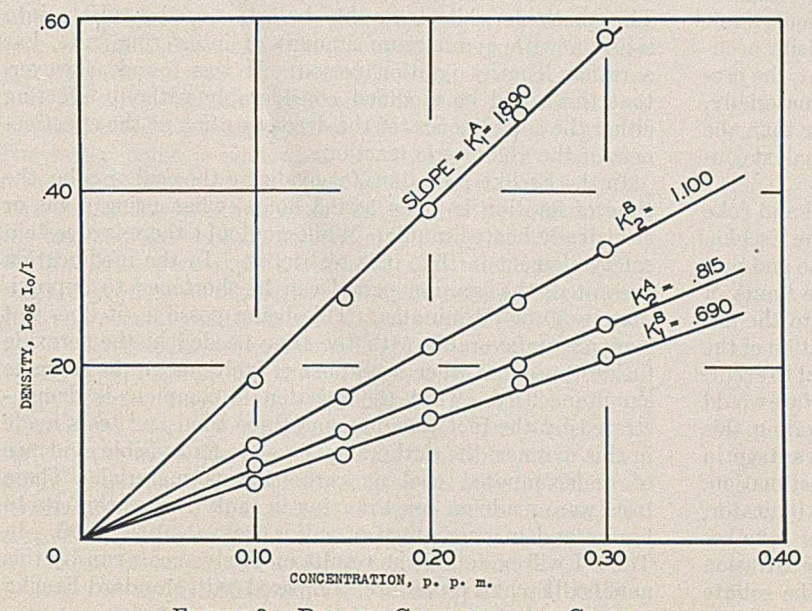

FIGURE 3. DENSITY-CONCENTRATION CURVES

Summary

One method of resolution of a two-component color system using a photoelectric filter photometer depends on the ability to isolate part of the absorption band of the desired component by means of a suitable filter. The other method depends on the differential separation of the absorption bands by means of two appropriately selected filters. The details for calculating the concentration of one desired component in unknown mixtures with second component are given.

Experimental evidence in support of these theoretical conclusions is offered in the case of the aluminum-iron-hematoxylin system. A calibration is given for this system, together with a table of experimental results on synthetic samples.

Some change in the concentration of reagents used in the hematoxylin method is recommended.

This method is offered for determining small

amounts of aluminum in natural waters in which only small amounts of iron occur.

The recent development of photoelectric grating spectrometers may be expected to extend the application of the principle herein described.

Acknowledgment

The authors wish to thank the J. T. Baker Chemical Company for the grant which facilitated this research and also express appreciation to the J. T. Brittingham Fund for special spectrophotometric equipment which was used in this work.

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Determination of Sulfur in Coal and Coke

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IN THE usual proximate analysis applied to coal and coke, the determination of sulfur is required. That it is the most time-consuming and from this standpoint the most bothersome detail, especially when reports are to be rendered quickly and accurately, is well known. The interest in more rapid procedures is, to a great degree, indicated by the extensive publication in the past, together with current contributions on the estimation of the sulfate ion. In practically every instance the time element is stressed in conjunction with accuracy, and endeavors are made to substitute a volumetric procedure in place of the lengthy gravimetric determination of barium sulfate.

While in many cases involving the evaluation of coal or coke only an approximation of this constituent is needed, in others a rather high degree of precision is essential. Commercial laboratories, receiving samples from a variety of sources, and industrial or control laboratories with exacting demands, find it necessary to incorporate in their routine only those procedures or methods that will be capable of giving results within the accepted tolerances. When reports are often expected to be completed within 3 or 4 hours, it is difficult if not impossible to combine this rapidity with the requisite accuracy. Dealing with the problem from this viewpoint, the procedures suggested which shorten the time interval materially, without affecting the precision to an extent greater than the permissible variations, should be of interest to the analyst concerned.

The three methods for determining sulfur in coal and coke now accepted as standard by the A. S. T. M. are the Eschka, peroxide fusion, and bomb washing (*1*). Much time and precaution are necessary to ensure results within the limits of allowable error, particularly if due regard is paid to the important steps in the precipitation, settling, and filtration of the barium sulfate, where most of the larger errors are apt to occur. The use of a volumetric procedure to estimate the sulfate would eliminate a great many of the difficulties surrounding this phase of the test, and would be of material advantage in formulating a faster and equally exact method of estimation. In adapting such a procedure, using an internal indicator, the means used to decompose or oxidize the coal or coke becomes an essential feature of the test. When the titration depends on the velocity of the reaction between the sulfate ion and barium chloride, the inclusion of certain salts or their degree of concentration in the solution of sulfate will prove troublesome. Under certain conditions, the reaction may be inhibited to the point of making the titration impractical, if not impossible.

Of the three standard procedures, the peroxide fusion method offers the quickest means of oxidizing the coal or coke and bringing the sulfate into solution in a condition ready for precipitation. Unfortunately, it contributes to the test solution an excessive amount of sodium chloride, and certain interfering ions.

The bomb washing method, while a desirable means of bringing the sulfate into solution comparatively free from interfering substances, would be prohibitive to many laboratories from an equipment standpoint, aside from the time factor involved in manipulating an oxygen bomb or bombs when a number of determinations are to be made. For tests made in conjunction with the calorific value, its use is probably warranted.

This would leave for consideration only the Eschka procedure as an ordinary means of decomposing the coal or coke. This method, while favorable to bringing the sulfate into solution with a minimum amount of interfering salts, has a rather lengthy ignition period. It was found, however, that this could be modified considerably without affecting either the completeness of the decomposition or the effectiveness of the volumetric reactions.

In the Eschka procedure for oxidizing the coal or coke, the time of ignition is given as 2.5 hours, when using a gas or electrically heated muffle. While no doubt there is a certain safety element in this, it is restrictive. In the modification presented, the ignition period can be shortened to approximately 50 or 60 minutes. This being possible, it does not compare unfavorably with the time needed in the peroxide fusion procedure when a number of ignitions are to be made simultaneously. That the reaction is complete is demonstrated by the fact that in some three thousand tests made in this manner, the authors have yet to find visible evidence of undecomposed coal or carbonaceous material. These tests were made on coals ranging in rank from anthracite to lignite and in percentage of sulfur from 0.40 to 15.00. In Table I will be found the results on twelve coals run by this modified Eschka procedure, compared with standard Eschka and peroxide fusion methods.

Modified Eschka Method

A 0.5-gram sample of the coal or coke prepared through a U. S. No. 60 sieve is thoroughly mixed with 3.0 grams of the regular Eschka mixture and 0.1 gram of potassium perchlorate (with anthracite and coke 0.2 gram). Shaking in an ordinary, glass-stoppered weighing bottle 50 mm. high is a satisfactory way of obtaining the intimate mixing necessary. The mixture is transferred to a crucible of about 15-cc. capacity (the use of a fire-clay annealing cup with a thin layer of Eschka mixture on the bottom is suggested to prevent the adhering of the contents to the sides and bottom after ignition), then packed or settled by tapping, and capped with about 0.5 gram of the Eschka mixture. The crucible is placed in a muffle furnace at 360° to 370° C. and the temperature is gradually raised to 530° C. in approximately 15 minutes, then to 760° C. in 10 minutes, and held at this point for 25 minutes (anthracite and coke may require an additional 10 or 15 minutes). If puffing or "blow outs" occur, they are likely to be due to the size consist of the Eschka mixture, rather than the rate of heating. Mixtures specially prepared to minimize this difficulty are commercially available from the Burrell Technical Supply Company, Pittsburgh, Penna.

After cooling, the contents of the crucible are transferred directly to an 11-cm. filter (Whatman No. 1 type), the crucible is rinsed, and the contents of the filter are washed with distilled water at almost boiling temperature until the filtrate amounts to approximately 150 ml. The filtrate is made slightly acid to methyl orange with dilute hydrochloric acid (1 to 5), 5 ml. of saturated bromine water are added, and the solution is boiled until no odor of bromine is apparent and the volume is between 35 and 50 ml. It is cooled to 20° C., a few drops of phenolphthalein indicator solution are added, and it is brought to a faint pink with dilute ammonia water (1 to 10). An equal volume of ethyl alcohol or specially denatured No. 30 is added. If legal restrictions make the use of these inconvenient, acetone or isopropyl alcohol can be used in the same manner. The solution is now ready for the volumetric determination of the sulfate.

A number of trials, using the method as outlined in the standard Eschka procedure, show that when the Eschka modification is used, blank corrections are unnecessary if the reagents are of tested purity, free from or very low in sulfur. The ignition, solution, filtration, and subsequent treatment are carried through and a definite amount of the standard ammonium sulfate is added and determined by the use of either sodium rhodizonate or tetrahydroxyquinone as indicator. This particular point must be determined in each case. Because of the nature of the test procedure and the color reaction, certain operators will find a small but significant blank correction essential.

For the titration of sulfate the use of either sodium rhodizonate or tetrahydroxyquinone as internal indicator was found possible. Schroeder (*4*), in a comprehensive survey of the methods that have been proposed for the estimation of sulfate, specifically dealt with the titration of sulfate, using both sodium rhodizonate and tetrahydroxyquinone. He gave preference to tetrahydroxyquinone, in that it could be used for direct titration and was the most satisfactory in solutions more dilute than 0.1 or 0.2 *N* in respect to sulfate. The latter point is somewhat at variance with what the authors have found in this investigation, for in working with coals containing less than 1 per cent sulfur, the normality of the test solution will be considerably below this limit, yet no difficulties were experienced with determinations in this range, using sodium rhodizonate, the end points being as distinct and accurate as those in solutions having the higher sulfate concentrations. The two indicators were found to give equally good results; the choice will rest with the individual analyst's preference for the color changes involved.

Schroeder (*4*), Mutschin and Pollak (*3*), and Kefeli and Berliner (*2*) have described the color reactions when using sodium rhodizonate; these are such as to make an indirect titration necessary. The change is from a red in the presence of an excess of barium salt to a pronounced canary yellow with excess of sulfate, the end point being distinct with both natural and artificial light. This latter point was instrumental in the choice of this indicator for routine determinations. As the reactions involved in the use of both indicators are nonreversible in the ordinary sense, an indirect titration can be considered an advantage, rather than a handicap. An approximation of the amount of sulfate present is obtained by observing the precipitation of barium sulfate, when an excess of barium chloride is being added. While sodium rhodizonate can be used in a saturated water solution, it is very unstable and must be freshly prepared every few hours. For convenience, a dry dispersion in ammonium chloride is used in a ratio of 1 to 400, prepared by grinding in a mortar 0.1 gram of the salt with 40 grams of ammonium chloride until an intimate mixture is obtained. This appears to be very stable; some has been stored as long as 6 months with no appreciable change in effectiveness. Tetrahydroxyquinone is marketed ready for use in a dispersed form, or can be prepared as above using potassium chloride.

Indirect Titration Using Sodium Rhodizonate

To the filtrate from the Eschka fusion, prepared as described and containing approximately 50 per cent of ethyl alcohol, a measured amount of an approximate 0.1 *N* solution of barium chloride, standardized gravimetrically, is added, sufficient to precipitate the sulfate with an excess of 3 to 5 cc. This excess can be estimated readily with a little practice. After allowing a minute or two for completing the reaction, 0.2 gram of the sodium rhodizonate–ammonium chloride indicator is added and thoroughly mixed by swirling. At this point the solution should have acquired a distinct red color, indicating an excess of barium chloride; if not, a measured quantity of barium chloride should be added, as well as more indicator. It is important that the indicator be added after the reaction is complete and an excess of barium chloride is present.

For the determination of the excess barium chloride, the solution is titrated with a standard solution of ammonium sulfate (adjusted to the barium chloride used in the precipitation). The back-titration can be made at a rate of about one drop per second with constant swirling, until there is a slight fading of the original red to a light pink; it is then slowed down to 5 or 7 drops a minute, until one drop produces a light orange tint. This indicates the end point, and the titration is stopped and the solution set aside. Within a minute or two, this will change to a

TABLE I. DETERMINATION OF SULFUR

Sample	Standard Eschka Gravimetric	Modified Eschka: Gravimetric	Modified Eschka: Titration with sodium rhodizonate	Modified Eschka: Titration with tetrahydroxyquinone	Peroxide Fusion Gravimetric
	%	%	%	%	%
A	0.78	0.79	0.82	0.76	0.81
B	0.94	0.91	0.92	0.93	0.93
C	1.26	1.28	1.22	1.15	1.15
D	1.38	1.38	1.36	1.42	1.42
E	1.38	1.40	1.43	1.46	1.42
F	1.40	1.33	1.38	1.43	1.41
G	1.43	1.48	1.46	1.51	1.35
H	1.46	1.42	1.43	1.48	1.50
I	1.80	1.78	1.71	1.91	1.78
J	1.88	1.82	1.82	1.96	1.96
K	3.30	3.28	3.18	3.32	3.27
L	4.37	4.46	4.30	4.34	4.20
13	..	1.01	1.00	1.04	..
14	..	1.81	1.72	1.80	..
15	..	6.36	6.32	6.42	..
16	..	1.50	1.52	1.44	..
17	..	2.08	2.00	..	..
18	..	1.92	2.00	..	..
19	..	1.78	1.71	..	..
20	..	3.09	3.00	..	..
21	..	0.95	1.00	..	..
22	..	0.60	0.56	..	..
23	..	1.82	1.76	..	..
24	..	3.36	3.46	..	..
25	..	0.79	0.82	..	..
26	..	1.15	1.17	..	..
27	..	0.80	0.80	..	..
28	..	3.32	..	3.46	..
29	..	1.78	..	1.82	..
30	..	1.66	..	1.66	..
31	..	1.86	..	1.88	..
32	..	2.04	..	2.03	..
33	..	1.93	..	2.06	..
34	..	1.09	..	1.14	..
35	..	1.31	..	1.30	..
36	..	2.08	..	2.16	..
37	..	1.73	..	1.72	..
38	..	0.92	..	0.92	..

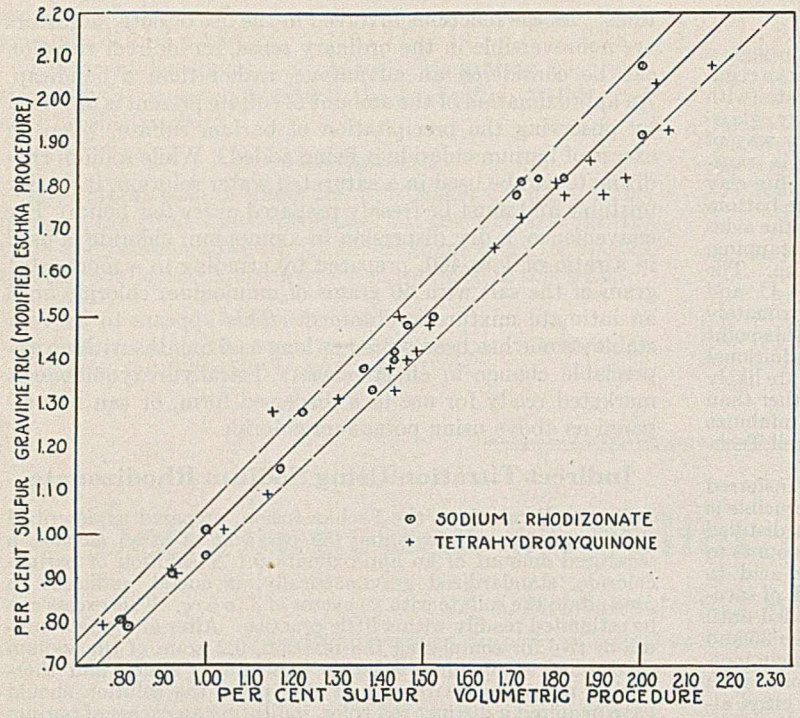

FIGURE 1. DEVIATIONS FROM GRAVIMETRIC PROCEDURE

pronounced canary yellow; if this does not occur, one more drop is usually sufficient. It is not possible to titrate directly to this yellow, as it invariably means an overrun introducing a significant error on the low side. While it is somewhat difficult to describe this end point exactly in terms of color, it becomes very apparent with a little practice, using known solutions. Where the color changes are not so sharp as might be expected, it is advisable to add from 0.2 to 0.5 gram of ammonium chloride salt.

In Table I, there are given the results of this procedure when compared with the gravimetric technique.

Direct Titration Using Tetrahydroxyquinone

In the use of tetrahydroxyquinone as an internal indicator, the procedure is very similar, except that the titration is completed using the standard barium chloride solution.

To the solution as prepared above about 0.2 gram of tetrahydroxyquinone (as obtained from W. H. & L. D. Betz Company, Philadelphia, Penna.) is added and thoroughly mixed. The solution should acquire a distinct yellow color, varying in some degree with the amount of sulfate present. The standard barium chloride is added dropwise fairly rapidly to the appearance of a brownish tint; this signifies the approach of the end point. The titration is slowed down and each drop well mixed throughout, until one drop produces a red or pink color throughout the solution, denoting the end point. When using solutions low in sulfate (less than 1.0 per cent sulfur in coal), more care must be exercised for, owing to a rather indistinct color change, there is a tendency to overrun.

Table I gives the results using this method compared with the gravimetric.

For both the sodium rhodizonate and the tetrahydroxyquinone determinations in Table I, the samples designated by letter were run under special conditions. The results on the numbered samples were obtained under routine procedure. Figure 1 is a graphical representation of the deviations from the gravimetric procedure of a number of typical determinations from Table I, in the range of sulfur from 0.70 to 2.10 per cent. The broken lines indicate the tolerance allowed. While the present standards state that agreement must be within 0.05 on percentages under 1.0 and 0.10 on those 2.0 and over, under these tolerances it is reasonable to calculate that between these points the allowable deviation will be 5.0 per cent of the total sulfur present. Examination of these data indicates that the points with but minor exceptions fall well within the limits allowable. These exceptions can hardly be considered serious because the method of plotting assumes absolute accuracy for the gravimetric determinations, whereas in fact there is an expected deviation within the standard limits for both methods.

Volumetric Procedure Using Peroxide Fusion Method

The sodium peroxide fusion method has the advantage of speed in the oxidation of the coal substance; however, a good deal of the time saved is subsequently lost by a necessary precipitation and filtration and a slowing down of the titration. It is possible to use this method in certain types of testing, if accuracy of the usual order is not necessary.

A 0.5-gram sample of the coal or coke is fused in the usual manner with about 12 grams of sodium peroxide, and the fusion product is dissolved in water, made slightly acid with hydrochloric acid, and boiled. While boiling, concentrated ammonia water is added to precipitate the iron, aluminum, etc., which interfere. The solution is filtered, the filtrate is concentrated to a volume of about 80 cc., and a few drops of phenolphthalein indicator solution are added. It is made just acid with dilute hydrochloric acid (1 to 5), and then brought to a faint pink with dilute ammonia water (1 to 10). It was found advantageous here to aliquot the solution and average the results of two titrations. After cooling, an equal quantity of ethyl alcohol is added, and from this point either sodium rhodizonate or tetrahydroxyquinone can be used.

The relatively large amount of sodium chloride in the solution presents some difficulties, not only slowing the reaction rate between barium chloride solution and sulfate but also exerting a bleaching or fading effect on the indicator. The titrating solution must be added slowly and carefully, allowing ample time for completing the reaction. If this procedure is prolonged, it may be necessary to add more indicator to overcome the color-fading action of the chloride. In Table II are given the results by this method, compared with a standard gravimetric estimation. These results are probably better than would be obtained under routine conditions.

TABLE II. DETERMINATION OF SULFUR

Sample	Standard Eschka Gravimetric	Peroxide Fusion Titration with Tetrahydroxyquinone
	%	%
1	0.78	0.86
2	1.38	1.44
3	1.40	1.45
4	1.42	1.38
5	1.42	1.51
6	1.46	1.40
7	1.46	1.52
8	1.87	1.96
9	3.34	3.22
10	4.34	4.16

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Recovery of Furfural from Aqueous Solutions

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IN GENERAL there are two methods for separating the components of a solution: distillation and extraction. The former method as described by Mains (*4*) has found extensive use commercially for the recovery of furfural from aqueous solutions. To the authors' knowledge no solvent extraction method has ever been proposed for this particular separation. In this paper an extraction procedure is described, and to give a comparison of the two methods the steam requirement for each has been calculated.

Materials and Procedure

The furfural was prepared by fractionating technical furfural three times through a 180-cm. (6-foot) Hempel column packed with 6-mm. glass Raschig rings. The pressure was maintained at 15 mm. of mercury, and discards of 15 per cent of the total volume were made at the beginning and end of each fractionation.

The ethyl acetate used was Baker's C. P. absolute.

The solubility data were obtained by titration in the following manner: Solutions of known concentration of two of the components were prepared and then titrated slowly with the third component until the cloud point was reached. This point was assumed to be the saturation point of the system, and inasmuch as comparatively large volumes of furfural, ethyl acetate, and water were employed a rather high degree of accuracy and reproducibility was attained. During the entire procedure the temperature was maintained at 25° C. in a constant-temperature bath.

The mutual solubility of furfural and water and of ethyl acetate and water at 25° C. was obtained from International Critical Tables (*3*).

TABLE I. SYSTEM FURFURAL–ETHYL ACETATE–WATER AT 25° C.

Furfural	Ethyl Acetate	Water
%	%	%
2.7	5.8	91.5
5.8	3.3	90.9
11.0	85.2	3.8
19.8	76.1	4.1
26.8	69.0	4.2
37.6	57.9	4.5
45.4	49.9	4.7
51.3	43.9	4.8
55.9	39.2	4.9
56.2	39.0	4.8
61.8	33.3	4.9
68.5	26.4	5.1
77.1	17.8	5.1
82.3	12.6	5.1
88.0	6.8	5.2

The data in Table I are presented in graphical form in Figure 1, the shaded areas denoting the region of complete miscibility and the blank region indicating compositions of the three components which separate into two liquid phases.

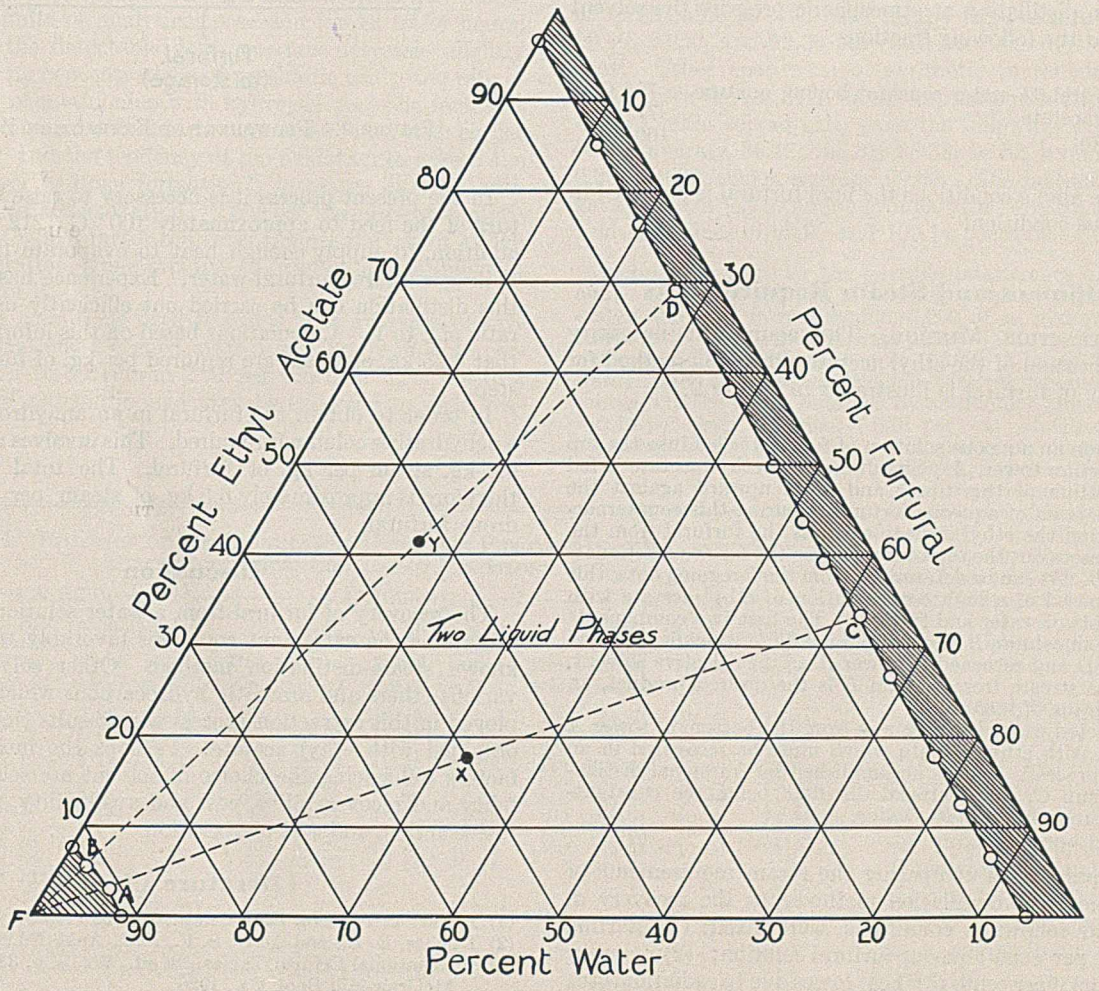

FIGURE 1. MISCIBILITY RELATIONSHIPS OF FURFURAL, ETHYL ACETATE, AND WATER AT 25° C.

To illustrate the method of reading Figure 1, refer to point X. This point represents a mixture comprising 50.0 per cent water (scale at base of triangle), 17.5 per cent ethyl acetate (scale at left side of triangle), and 32.5 per cent furfural (scale at right side of triangle).

The focal point, F, was located in the following manner after Figure 1 had been plotted: Two known mixtures of furfural, ethyl acetate, and water (represented by points X and Y in Figure 1) were prepared and allowed to stand at 25° C. until equilibrium conditions were attained. Each mixture formed two layers which were separated and analyzed for furfural by the Hughes-Acree method (*2*). From the results obtained, points were located on the water layer and solvent layer boundary lines. Composition X separated to form two layers, the compositions of which are represented by points A and C. Similarly composition Y formed two phases of compositions B and D. Lines were then drawn through points DYB and CXA, focus F being established at the point of intersection.

Any mixture of composition lying within the two-phase area (blank region in Figure 1) on a line extended through F will separate into two layers represented by the compositions where this line intersects the boundary curves. Because of the difference in the densities of the two layers, however, one cannot read directly from the graph the per cent of furfural that is extracted from a given aqueous solution of the latter. This can be determined experimentally or by the mathematical method described by Evans (*1*). Application of the Evans method shows that one extraction of a 7 per cent aqueous solution of furfural with an equal weight of pure ethyl acetate removes 93.5 per cent of the furfural from the water layer. A second extraction of the water layer would remove over 90 per cent of the remaining furfural.

In the laboratory, using a 240-cm. (8-foot) extraction column packed with 6-mm. porcelain Raschig rings, a 7 per cent aqueous solution of furfural was extracted countercurrently with an equal weight of ethyl acetate. Analysis of the solvent layer indicated that 99.9 per cent of the furfural had been extracted. On distillation at atmospheric pressure the solvent layer yielded the following fractions:

1.	Ethyl acetate–water constant-boiling mixture	70° C.
2.	Ethyl acetate	77° C.
3.	Furfural	160° C.

Under the above conditions the final furfural is obtained in an anhydrous condition.

Equipment and Steam Requirements

For Extraction Method. The equipment necessary for the application of the ethyl acetate extraction method for the recovery of furfural is illustrated in Figure 2.

In operation an aqueous solution of furfural is led into the top of the extraction tower, A. Simultaneously ethyl acetate is fed into the bottom of this tower and flows upward against the descending stream of aqueous furfural. During this countercurrent separation the ethyl acetate extracts the furfural from the water and passes out the top of tower A as the feed to fractionating column B. As can be determined from the foregoing data, this feed is composed of a high concentration of ethyl acetate with lesser amounts of water and furfural. The first two components are removed in column B as overhead distillate which is collected in reservoir D and returned to tower A as the selective solvent. The bottoms stream from column B is the desired product, an anhydrous grade of furfural.

The extracted water layer issuing from the bottom of tower A is saturated with ethyl acetate which must be recovered in an economical process. This is accomplished by fractional distillation in column C, the overhead distillate being the constant-boiling mixture ethyl acetate–water.

For the purpose of comparing the steam requirements of the extraction and distillation methods for the recovery of furfural the following conditions were fixed: (1) starting material, 7 per cent aqueous furfural solution; (2) recovery of furfural 99.9 per cent; (3) heat losses due to radiation, etc., 10 per cent; (4) savings by heat exchangers omitted.

We shall assume that equal weights of feed and of ethyl acetate are used in tower A (Figure 2). The heat requirements in column B were calculated to be 3.4 kg. of steam per kg. of anhydrous furfural. It is necessary, however, to add to this the heat necessary to recover the ethyl acetate in column C, which raises the total steam expenditure to approximately 5 kg. per kg. of anhydrous furfural.

For Steam Distillation Method. The equipment necessary for this process has been described by Mains (*4*). However, the steam requirement in the authors' case differs from that of Mains, because of the use of a higher feed concentration and a different reflux ratio.

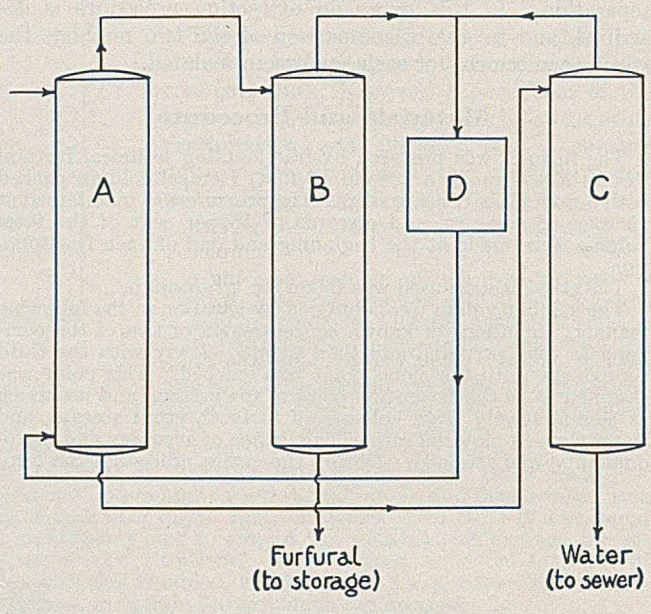

Figure 2. Flowsheet of Extraction Plant

In the present process it is necessary to raise the temperature of the feed to approximately 100° C. (212° F.) and, in addition, to supply enough heat to evaporate the constant-boiling mixture, furfural-water. Experience has shown that this distillation can be carried out efficiently using a reflux ratio of 2 to 1. Calculations based on this information show that 6.25 kg. of steam are required per kg. of furfural in this step.

In order to obtain the furfural in an anhydrous condition a dehydrating column is required. This involves an additional 0.2 kg. steam per kg. of furfural. The total requirement therefore is approximately 6.5 kg. of steam per kg. of anhydrous furfural.

Discussion

The recovery of furfural from a water solution using ethyl acetate as the extractant compares favorably with the well-known steam-distillation method. Other solvents include various ethers and aromatic hydrocarbons which can be employed in this extraction process with results similar to those obtained with ethyl acetate. Perhaps the most important factors influencing the choice of solvent are selectivity, stability and recoverability, cost and availability, toxicity, corrosive action, and cost of operation.

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Determination of Methionine in Certain Mixtures[1]

A Precision Method

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THE ready oxidizability by hydrogen peroxide of the amino acid methionine to the sulfoxide level (*4*) suggests the possibility of analytical utilization of this reaction. It has been shown that it can serve for the determination of methionine in crude protein hydrolyzates but that the accuracy is impaired by the presence of interfering substances (*3*). The present communication has as its object the determination of suitable conditions for, and the precision attainable in, the application of peroxidimetry to the assay of methionine preparations, and the investigation of the oxidizability of other amino acids under comparable conditions.

Experimental

Since the time required for the completion of the methionine oxidation decreases with increasing acid concentration (*3*), and since among the mineral acids perchloric acid appears to be the one least likely to interfere in oxidations and reductions (*4*), the course of the reaction was carefully followed in perchloric acid solutions of 4 *M*, 2 *M*, and 1 *M* concentration. Table I shows the results obtained. The conclusions suggested by the data are: the freshly prepared solutions of hydrogen peroxide in aqueous perchloric acid are, apart from possible minor adjustments during a brief initial period, stable within the limits of analytical precision for 12 to 24 hours; the rate of the detectable decomposition decreases slightly with increasing concentrations of perchloric acid; the rate of the reaction of methionine with hydrogen peroxide increases with increasing acid concentration, in accordance with earlier findings (*3*); and the tendency of the oxidation to exceed the sulfoxide level (sulfone formation?) increases likewise with increasing acid concentration. Thus for the present analytical purpose the last factor militates against the use of a high acid concentration, while rapidity of reaction and stability of peroxide favor it.

Under the conditions used it seems that averages of several determinations obtained after 3 to 7 hours in 1 *M* perchloric acid, 1 to 3 hours in 2 *M* perchloric acid, or 0.5 to 2 hours in 4 *M* perchloric acid may be expected to yield reasonably precise values for the peroxide consumed in the formation of sulfoxide from methionine. The reaction follows substantially a bimolecular course, and other determinations have shown that, in harmony with the bimolecular reaction formula, more time or a higher peroxide-methionine ratio is required for completion of the reaction when the absolute concentrations are lower.

The data of Table I give the following results for the purity of the methionine used: from the average of the figures obtained in 4 *M* perchloric acid between 25 and 120 minutes, 99.62 ± 0.12 per cent, and from that of the 2 *M* perchloric acid figures between 50 and 180 minutes, 99.60 ± 0.03 per cent. These figures were checked by a series of single titrations on individually weighed samples, as follows:

About 100 mg. of methionine are weighed into a 250-cc. glass-stoppered Erlenmeyer flask. After addition of 10 cc. of 0.08 *M* hydrogen peroxide in 1 *M* perchloric acid the sample is dissolved by gentle swirling. After 4 or 5 hours 20 cc. of a solution of 5 millimoles of potassium iodide and 0.1 millimole of ammonium molybdate are added and the liberated iodine is at once titrated with thiosulfate. A control identical except for the omission of methionine is treated in the same manner.

The following results were obtained on the same specimen of methionine as was used in the experiments of Table I: 99.49, 99.50, and 99.36 per cent; average 99.45 ± 0.06 per cent. Other specimens of synthetic methionine, some of which were the result of an attempted purification by way of the insoluble copper salt, gave the following values: 98.39, 98.45, average 98.42; 98.31, 98.38, 98.52, average 98.40 ± 0.08; 99.60, 99.78, average 99.69. The last-named sample gave by titration of amino groups with "aceteous" perchloric acid (*2*) values of 99.90 and 100.14 per cent.

In order to consider the possible interference of some other amino acids 4-cc. samples of a freshly prepared solution approximately 0.073 *M* in methionine, 0.090 *M* in hydrogen peroxide, and 2 *M* in perchloric acid were pipetted into flasks containing 1 millimole of another amino acid. After 2 hours the solutions were titrated as usual, with the following results, expressed as percentages of the values obtained by methionine alone: *dl*-alanine, −0.10 per cent; *dl*-serine, +0.01 per cent; *dl*-threonine, +0.09 per cent; *dl*-phenylalanine, −0.02 per cent; *l*-tyrosine, −0.05 per cent; *l*-proline, −0.10 per cent; *l*-hydroxyproline, +0.02 per cent; *l*-lysine dihydrochloride, +0.20 per cent; *l*-histidine monohydrochloride, −0.10 per cent; *l*-arginine monohydrochloride, +0.09 per cent; *l*-aspartic acid, −0.02 per cent; *l*-glutamic acid, −0.06 per cent; *l*-tryptophane, +2.1 per cent; *l*-cystine, +1.8 per cent.

Apparently the ordinary amino acids, except to a small extent tryptophane and cystine, as well as cysteine (*3*), do not consume any hydrogen peroxide under the present conditions. This circumstance should recommend the present method for the detection of methionine present as a contaminant in preparations of leucine of natural origin (*5*).

[1] Methionine Studies V.

TABLE I. REACTION OF METHIONINE AND HYDROGEN PEROXIDE IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF PERCHLORIC ACID

4 *M* $HClO_4$				2 *M* $HClO_4$				1 *M* $HClO_4$			
	H_2O_2 (blank)	H_2O_2 (+ Methionine)			H_2O_2 (blank)	H_2O_2 (+ Methionine)			H_2O_2 (blank)	H_2O_2 (+ Methionine)	
Time	found	Found	Consumed	Time	found	Found	Consumed	Time	found	Found	Consumed
Min.	*mM./l.*	*mM./l.*	*mM./l.*	*Min.*	*mM./l.*	*mM./l.*	*mM./l.*	*Min.*	*mM./l.*	*mM./l.*	*mM./l.*
5	83.05	8.66	74.39	5	...	12.95	...	100	84.04	10.63	73.41
10	(83.06)[a]	8.52	74.54	10	83.65	12.58	71.07	150	83.65	10.38	73.27
25	(83.10)[a]	8.44	74.66	25	83.59	12.21	71.38	200	83.74	10.24	73.50
35	(83.12)[a]	8.44	74.68	50	83.63	11.91	71.72	250	83.61	10.22	73.39
60	83.16	8.40	74.76	75	83.59	11.91	71.68	300	83.67	10.28	73.39
120	83.20	8.34	74.86	125	83.59	11.91	71.68	350	83.69	10.28	73.40
1100	83.16	7.18	75.98	180	83.59	11.91	71.68	400	83.63	10.28	73.35
2500	83.02	5.94	77.08	1200	83.50	11.45	72.05	450	83.63	10.28	73.35
5400	81.89	..	...	2600	83.24	10.76	72.48	1500	83.52	10.02	73.50
7200	81.28	..	...	5500	82.63	9.48	73.15	..	...	...	...
8700	81.16	..	...	7200	81.70	...	...	..	...	...	...
			75.01[b]				71.98[b]				72[c]

[a] Interpolated values.
[b] Methionine calculated according to weight used.
[c] Approximate amount of methionine (not weighed precisely).

The applicability of the present method to various other situations, and the accuracy possible, will have to be ascertained from case to case. Grace Medes of this institute has successfully adapted the method to the determination of the fate of methionine in tissue extracts; no difficulty was experienced in determining with an accuracy of 0.5 per cent amounts of the order of 0.25 millimole of methionine, dissolved in 5 cc. of buffer containing about 25 mg. of liver slices.

Summary

The purity of methionine can be determined with an accuracy of ±0.1 per cent by oxidation with hydrogen peroxide under specified conditions. The principle of this method is applicable to the determination of methionine in certain other analytical situations, since other natural amino acids, except tryptophane, cysteine, and cystine to a small extent, do not seem to interfere. Data on the stability of hydrogen peroxide in 1 to 4 molar perchloric acid solutions are included.

A 0.21 *M* hydrogen peroxide solution in 1.25 *M* perchloric acid was prepared from Merck's Blue Label hydrogen peroxide which contains no preservative. Ten- or 20-cc. portions were transferred to 25- or 50-cc. volumetric flasks and the acidity was adjusted with perchloric acid so that the final acid concentrations would be 4.0, 2.0, and 1.0 *M*, respectively, when the solutions were diluted to the mark with water. In similar flasks weighed amounts of synthetic *dl*-methionine were dissolved in water and combined with hydrogen peroxide and perchloric acid in such a manner that the acid concentration and the initial amount of peroxide were identical with the contents of the corresponding blank solutions. The molar amount of methionine was 10 to 15 per cent less than that of the peroxide. At intervals 3- or 5-cc. aliquots of the completed solutions were pipetted into an Erlenmeyer flask, 20 cc. of a solution containing 5 millimoles of potassium iodide and 0.1 millimole of ammonium molybdate were added (*3*), and the liberated iodine was immediately titrated with 0.025 *N* thiosulfate using starch indicator.

The thiosulfate was standardized against copper (*1*). Pipets and flasks used in the experiments with 4 *M* and 2 *M* perchloric acid were carefully calibrated.

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Determination of Organic Sulfur in Combustible Gas

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Purified air is used to burn the gas in a special burner, the sulfur dioxide resulting from the combustion of the gas is absorbed in sodium hypobromite solution, and the sulfate in this solution is determined turbidimetrically. The method is adapted to the determination of less than 0.2 grain (12.96 mg.) of sulfur per 100 cu. feet (283.2 × 10^4 cc.) of gas.

A NUMBER of methods for the determination of organic sulfur in gas have been described (*1–4*, *6*), but none is applicable to the analysis of hydrocarbon gas containing sulfur to the extent of 0 to 0.2 grain (12.96 mg.) per 100 cu. feet (283.2 × 10^4 cc.). Lieber and Rosen (*3*) have obtained excellent results by their method on gas containing as little as 0.6 grain of sulfur per 100 cu. feet. However, this laboratory has found that the time involved in burning a sufficient quantity of gas to give a weighable amount of barium sulfate prohibits the use of this method for routine analyses when quick results are desired.

The method developed by this laboratory has filled the demand for a thoroughly reliable, relatively rapid routine analysis of combustible gases for organic sulfur. It is to some extent a modification of existing methods and has been adapted to the determination of sulfur of the order of 0 to 0.2 grain per 100 cu. feet.

The method uses purified air (*7*) to burn the gas in a specially designed burner. The sulfur dioxide resulting from the combustion of the sulfur compounds is absorbed in sodium hypobromite solution. The sulfate in this solution is subsequently determined by the turbidimetric method (*5*) according to a modification previously described (*7*).

Analysis is made on gas from which all hydrogen sulfide has been removed by a suitable scrubbing procedure, such as passing the gas several times through a 20 per cent solution of lead acetate containing 5 per cent acetic acid or neutral cadmium sulfate solution.

Purification of Air

The air used for combustion is first filtered and dried by passing it through a tube containing a section of activated charcoal and then a section of soda lime. It is next passed through a copper spiral made from copper tubing 0.234 cm. ($^3/_{32}$ inch) in outside diameter with 0.117-cm. ($^3/_{64}$ inch) wall, maintained at a temperature of 540° C. (1000° F.) by means of a muffle furnace. The spiral is made up of 15 to 20 coils approximately 3.75 cm. (1.5 inches) in diameter. This arrangement permits very good heat transfer, and can be used at this temperature for many months without replacement. Higher furnace temperatures, however, cause the copper tubing to deteriorate fairly rapidly. It is important to subject the copper tubing to a preliminary ignition period at 1000° F. while a stream of purified air is passed through it to remove any sulfur compounds in the tubing.

After leaving the furnace, the copper tubing is spiraled and submerged in a water bath. The air is scrubbed first with 2 per cent sodium hydroxide–2 per cent bromine solution and then with 5 per cent sodium hydroxide solution. It is advisable to select for this purpose gas washing bottles which do not produce a fine mist of sodium hydroxide, as this will carry through to the burner and produce a yellow sodium flame.

From the gas scrubbing bottles the air is passed into a pressure reservoir from which a number of air take-offs can be made. This reservoir permits burning the gas with a steady, nonflickering flame. One of the take-offs from the reservoir leads to a manifold, from which in turn a number of leads can be taken. These are used for maintaining a sulfur-free atmosphere over the evaporating acidified sodium hypobromite solutions.

All rubber tubing and rubber stoppers used must first be heated for some time in strong caustic solution.

Gas Burner and Absorber

Although gas burners of the general type used in this work have been described, considerable difficulty encountered in their use necessitated developing the present burner and absorber (Figure 1). Its chief advantages are:

The ground-glass joint permits the burner tip to be easily and quickly centered in the chimney. This prevents smoking the chimney and increases gas combustion rate.

Incorporation of the chimney and absorber into one piece considerably reduces the number of parts which must be washed, thus speeding up the operation and reducing possibilities of contamination.

Rate of combustion of gas can be closely controlled by adjustment of stopcocks.

Gas can be made to burn with a clean blue flame even at the maximum burner capacity of 10 liters of gas per hour.

Elimination of a condensate trap is made possible because of the small sample of gas necessary to yield accurate results.

All rubber connections between burner and absorber are eliminated.

Total manipulative time consumed should not exceed 80 minutes per single determination made.

Reagents

A solution of 2 per cent sodium hydroxide and 2 per cent bromine in distilled water is used as absorbing liquid. Fifteen milliliters of this solution and 15 ml. of distilled water are used in each absorber.

Five per cent sodium hydroxide solution is used as a scrubbing liquid. The turbidimetric determination of sulfur requires 1 *N* hydrochloric acid, a 20 per cent solution of sodium hydroxide, an alcohol-glycerol solution (67 per cent by volume of ethyl alcohol and 33 per cent glycerol U. S. P.), and barium chloride dihydrate (20- to 30-mesh).

Reagents should be periodically tested for sulfate, especially if used over an extended period. Because 20 per cent sodium hydroxide solution reacts with glass and produces a turbid solution, which cannot be completely cleared by filtration, it is advisable to make up only small amounts of this reagent and not let it age. A blank determination is made on the reagents involved.

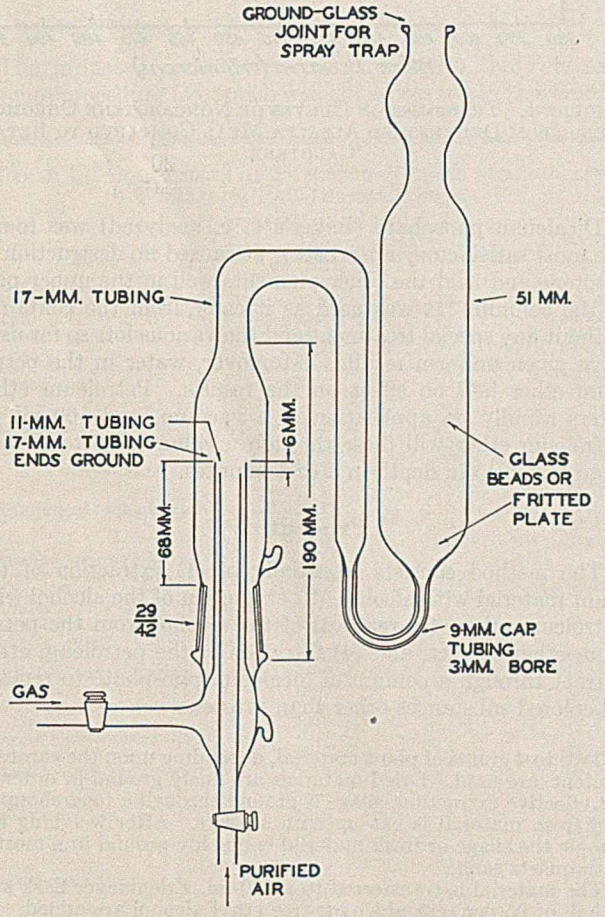

FIGURE 1. BURNER AND ABSORBER FOR DETERMINATION OF SULFUR IN GAS

Procedure

With the burner inserted in the chimney, adjust the flow of air to the proper velocity. Remove the burner and light the gas with an alcohol lamp. Adjust the flow of gas so that it burns with a clean blue flame.

Gradually reinsert the burner in the chimney. Doing this too quickly extinguishes the flame; if this occurs, care must be taken to sweep the apparatus free from gas before reignition. Suction on the absorber may be used to avoid extinguishing the flame, but the authors here found this to be unnecessary.

After sufficient sample has been burned (a 10-liter sample is sufficient for gas having 0.1 to 0.2 grain of sulfur per 100 cu. feet), transfer the absorbing solutions of the blank and the sample to 500-ml. Erlenmeyer flasks. Rinse the apparatus several times with distilled water. The final total volume should be about 200 ml. Acidify with 15 ml. of *N* hydrochloric acid. Stopper both flasks with 2-hole rubber stoppers having two lengths of small glass tubing projecting 2.5 cm. (1 inch) into the flask. One length of tubing is straight, whereas the second and longer piece is bent in a downward direction. Attach leads from the supply of purified air to the short tubing and begin evaporation of the solutions on a hot plate. Tests have shown it to be absolutely essential that these solutions be evaporated in an atmosphere free from contaminating sulfur compounds.

When the solutions have been concentrated to about 10 ml., filter through a Whatman's No. 40 filter paper into 50-ml. volumetric flasks, washing the Erlenmeyer flasks several times with distilled water. Make the filtrates slightly alkaline to phenolphthalein with 20 per cent sodium hydroxide, exactly neutralize with *N* hydrochloric acid, and add 3 ml. in excess. Make solutions up to the mark.

Follow with the determination of sulfate in these solutions, using the Betz-Hellige turbidimeter (*5*).

Initial turbidity should be negligible in all cases, and the blank should be very small in comparison with the sample being analyzed.

Results

The following data are typical of results which can be obtained by this procedure. The groups of two represent duplicate determinations simultaneously made on separate instruments, using two samples of gas of identical composition. Figures are expressed in terms of grains of sulfur per 100 cu. feet:

0.00539	0.0639	0.128	0.143	0.204
0.00503	0.0644	0.130	0.143	0.204
0.0207	0.0692	0.130	0.143	0.207
0.0225	0.0692	0.130	0.148	0.211
0.0521	0.0716	0.130	0.173	0.215
0.0526	0.0736	0.135	0.178	0.215
0.0580	0.126	0.137	0.204	1.92
0.0586	0.130	0.140	0.204	1.92

Values of 0.155 and 0.151 grain of sulfur per 100 cu. feet compare favorably with the value of 0.143 obtained on analysis of the same gas using the method of Lieber and Rosen (*3*).

Acknowledgment

The assistance of J. E. Moore is acknowledged in the glass blowing involved in the construction of the burner and absorber described.

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Determination of Carotene in Plant Material

Dicalcium Phosphate as an Adsorbent

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DURING the past few years considerable interest has been aroused in an effort to find a suitable method for the determination of carotene in feces and in plant material which has been stored as hay or made into silage. Apparently when plant material has been exposed to these conditions certain noncarotene chromogens develop which remain in the petroleum ether phase when carotene is determined by the Willstätter-Stoll method or its modifications (*1*). These noncarotene chromogens are therefore determined as carotene.

Hartman and co-workers (*3*) noted this discrepancy in pulverized alfalfa meal when comparing the values obtained biologically and chemically. Later Wiseman and associates (*10*) found noncarotene chromogens amounting to 11 to 32 per cent in the petroleum ether extract where the Willstätter-Stoll method of separation had been used. These chromogens were removed by filtering through a Tswett column of specially prepared magnesium oxide. Quackenbush and co-workers (*6*) found that when fresh plant material is subjected to mineral acids, as in the A. I. V. method of silage making, noncarotene chromogens develop which remain in the petroleum ether phase. Later work by Hegsted, Porter, and Peterson (*4*) showed that these chromogens could be removed by extracting the petroleum ether phase with diacetone.

Whitnah *et al.* (*9*) reported the excretion of over 100 per cent of the carotene fed to cows, which was probably due to yellow chromogens not carotene. Fraps and co-workers (*2*) found that the excrement from rats and chickens fed on rations practically free from carotene contains a yellow pigment not carotene.

It seems apparent that carotene in plant material, other than in the fresh state, cannot be accurately determined by the Willstätter-Stoll procedure or its modifications.

Wiseman and associates (*10*) used a specially prepared magnesium oxide in a Tswett column to remove the noncarotene chromogens from the petroleum ether extract but found it necessary to carry out the procedure at a reduced temperature and in an atmosphere of nitrogen which complicates the determination considerably. Fraps, Kemmerer, and Greenberg (*2*) shook the petroleum ether extract with a small amount of specially prepared magnesium carbonate. The use of diacetone as outlined by Hegsted, Porter, and Peterson (*4*) for silage did not remove all the noncarotene chromogens when used with extracts from hay in the author's laboratory.

Some time ago search was started in this laboratory for an adsorbent which would remove all pigments except carotene from the petroleum ether extract, would require no special preparation and no special precautions in carrying out the procedure, and would not change on standing in the laboratory. After trying many materials dicalcium phosphate was found to possess these qualifications.

Choice of Adsorbents

Dehydrated alfalfa leaf meal was extracted with petroleum ether and the extract passed through a Tswett column composed of the material being studied. By this procedure those adsorbents which held up the chlorophyll and carotenoid pigments with the exception of carotene could be ascertained and the rate of filtration could be observed.

The next step was to determine whether the adsorbent destroyed any carotene. This was done by passing a carotene solution through a column of the adsorbent and determining the amount of carotene before and after. This procedure was repeated several times with the same carotene solution, using fresh adsorbent each time. The column was not cooled nor placed in an atmosphere of nitrogen.

At first calcium carbonate was thought satisfactory, since it had been used for such a purpose in qualitative work. However, certain lots of calcium carbonate adsorbed or changed some of the carotene, others permitted filtration only at an exceedingly slow rate, and as a whole they varied considerably in their ability to hold up the noncarotene pigments.

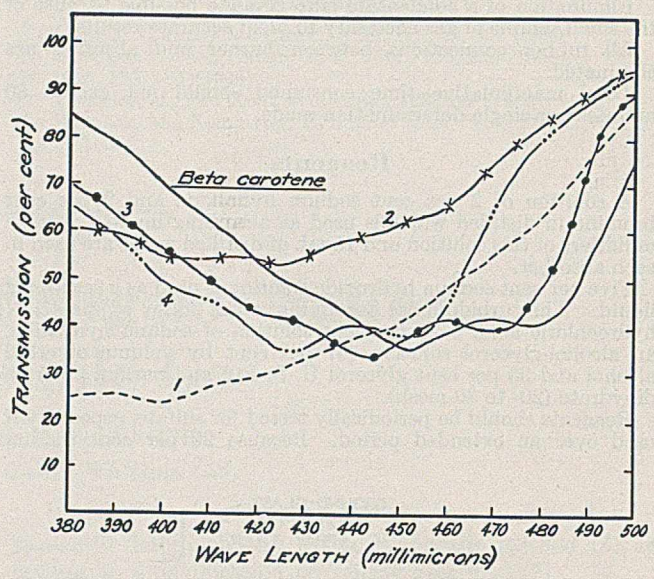

FIGURE 1. TRANSMISSION CURVES OF NONCAROTENE CHROMOGENS FROM DEHYDRATED ALFALFA MEAL COMPARED TO BETA-CAROTENE

Dicalcium phosphate (Baker's C. P. analyzed) was found the most satisfactory adsorbent. It caused no destruction of carotene and held the other pigments well in the upper part of the column. It was used as it came from the container without any special treatment and the various lots so far used have given uniform results. Moreover, water in the petroleum ether had no effect on the results. Petroleum ether filters rapidly on application of a vacuum, and 100 ml. of petroleum ether will filter through a column 7.5 to 10 cm. (3 to 4 inches) in depth in 3 to 4 minutes.

Procedure

The method consists essentially of (1) extraction of the plant material with alcohol, (2) extraction of the alcohol with petroleum ether, (3) removal of the alcohol from the petroleum ether extract, and (4) filtration of the petroleum ether extract through a column of dicalcium phosphate to remove all colored substances other than carotene.

Two to 4 grams of plant material, depending upon the carotene content, are used. Dried materials are finely ground in order to get effective extraction, silage is ground through a food chopper, and fresh material is cut up with scissors. After weighing the sample the silage or fresh material is quickly ground in a mortar with quartz sand.

The material is transferred to a 250-ml. Erlenmeyer flask and 50 ml. of 95 per cent aldehyde-free ethyl alcohol are added. In the case of the silage and fresh material, the alcohol is used in the grinding process and transfer.

The sample is then refluxed for 30 minutes on a water bath and cooled, the alcohol extract is decanted into a 500-ml. separatory funnel, and 25 ml. of alcohol are added to the residue; it is then refluxed for another 30-minute period and the second extract is decanted into the first. The residue is further extracted with petroleum ether and sufficient alcohol is added to keep the residue dispersed. Twenty-five milliliters of alcohol are generally used in this process, making a total of 100 ml., and about 50 ml. of petroleum ether. Sufficient water is then added to the extract to make the alcohol concentration 80 per cent.

The lower layer is separated from the upper layer, after which it is extracted three more times with 30-ml. portions of petroleum ether. This does not extract all the color from the alcohol phase, but as practically all the carotene is removed by the first two extractions there is no need for further extraction.

All the alcohol must be removed from the petroleum ether extract, otherwise all the other pigments will pass through the adsorbent with the carotene. For this reason the petroleum ether extract is then washed 6 or 7 times with 100-ml. portions of tap water, and concentrated to about 20 to 25 ml. in an Erlenmeyer flask by application of vacuum and hot water.

The tubes used for the dicalcium phosphate column can easily be made from ordinary test tubes approximately 19 cm. long and 2.2 cm. in diameter, by blowing out the bottom and attaching a small piece of glass tubing at the end. The glass tube is supported by a 250-ml. Erlenmeyer flask, held by a clamp, to catch the filtrate. A small piece of cotton is used to plug the small opening in the lower end of the tube, and a larger piece is placed on top. The tube is filled a little over half full with dicalcium phosphate, which is packed lightly by application of vacuum from an ordinary water pump and by use of a small plunger made with a glass rod and a cork of the proper size. A small amount of anhydrous sodium sulfate is placed on top of the phosphate, so that the tube is about half filled by the column.

Thirty milliliters of petroleum ether are run into the column, followed by the extract, concentrated to 20 to 50 ml., and 60 more ml. of petroleum ether. The various pigments are adsorbed in bands, while the carotene is washed on through the column. The carotene filtrate is then concentrated if necessary, and made up to volume, and the carotene concentration is determined. A photoelectric colorimeter was used in this laboratory.

Comparison with Peterson-Hughes Method

The proposed method was compared with the Peterson-Hughes procedure (*5*), which was already in use in this laboratory. For this comparison samples of fresh green material, grass and legume silages, stored hays, and bovine feces were analyzed by the two methods. The results of these determinations are shown in Table I.

In order to follow the progress of the development of the noncarotene chromogens a sample of dehydrated alfalfa leaf meal was obtained on which the two methods were in agreement as to the carotene content. This meal was then placed on the table and exposed to air in the laboratory; samples were analyzed at intervals to obtain the difference in results given by the two methods. The results are shown in Table II.

Table I. Comparison of Peterson-Hughes Method and Dicalcium Phosphate Column

Sample	Micrograms of Carotene per Gram of Material: P-H method	$CaHPO_4$ column	Difference %
Green plants			
Alfalfa	73.5	72.5	1.4
Rye	92.5	92.5	0.0
Alfalfa	104.0	105.0	0.0
Rye	100.0	97.5	2.5
Dandelion leaves	87.5	85.5	2.3
Quack grass	121.0	124.0	2.5
Stored hays			
Alfalfa hay	30.0	24.0	20.0
	38.0	30.0	22.0
	11.7	9.0	23.0
	23.0	20.0	13.0
	82.5	71.0	14.0
Alfalfa meal dehydrated	130.0	115.0	11.5
	126.0	110.0	12.7
	120.0	91.5	23.8
Grass and legume silages			
Brome silage, H_3PO_4 preservative	54.0	52.5	2.8
Alfalfa brome silage, H_3PO_4 preservative	52.5	47.5	9.5
Alfalfa silage, H_3PO_4 preservative	70.0	54.3	15.3
Alfalfa silage, no preservative	32.4	27.0	16.8
	14.0	7.8	44.2
Soybean silage, no preservative	29.3	23.3	20.3
Sweet clover silage, no preservative	15.0	9.7	35.5
Brome silage, no preservative	33.3	27.0	18.9
Bovine feces			
Winter feed	49.6	36.4	25.6
	60.0	45.0	25.0
	51.7	45.7	13.5
	87.0	81.0	6.8
Summer pasture	91.5	72.5	21.6
	91.0	82.5	9.3
	111.0	195.0	14.4
	112.8	96.8	14.2

Table II. Comparison of Peterson-Hughes Method with Proposed Method

(Using a sample of dehydrated alfalfa leaf meal exposed to laboratory temperature)

Date	Micrograms of Carotene per Gram of Meal: P-H method	$CaHPO_4$ column	Difference, %
11-4-39	145	143.5	1.0
11-9-39	140	125.0	10.7
11-24-39	97.5	86.0	11.7
11-30-39	83.5	71.5	14.4
12-7-39	77.5	65.0	16.1
12-15-39	69.0	55.0	20.2
12-22-39	65.0	46.5	28.9
12-28-39	59.5	44.5	25.2
1-4-40	50.0	37.5	25.0
1-19-40	40.0	30.0	25.0
2-19-40	30.0	19.5	35.0
2-29-40	23.0	15.3	33.5
3-9-40	22.0	13.3	39.6
3-15-40	21.6	14.0	38.5
3-28-40	15.3	10.0	34.7
4-4-40	13.2	9.2	30.4
4-11-40	11.7	8.2	30.0
4-18-40	10.3	7.0	33.3

Transmission Curves of Noncarotene Chromogens

Dehydrated alfalfa meal known to contain the noncarotene chromogens was extracted using the Peterson-Hughes procedure. The final extract was washed with water and the noncarotene chromogens were isolated on columns of calcium carbonate, dicalcium phosphate, and magnesium oxide. The columns of adsorbent were removed from the tubes and cut into sections, each section containing a different pigment. The sections were placed in Skellysolve B and the pigment was eluted by addition of a small amount of methyl alcohol. Transmission curves, as shown in Figure 1, were then made of each pigment and pure beta-carotene with a photoelectric spectrophotometer (manufactured by the Central Scientific Company, Chicago, Ill.).

As far as could be determined, the noncarotene chromogens of this sample of meal consisted mainly of four different pigments, numbered for convenience according to the order in which they appeared from top to bottom on the dicalcium phosphate column.

Pigment 1 was difficult to work with, as it seemed to undergo some change while on the column; on continued washing with the solvent it seemed to disappear gradually. It had a greenish-yellow color on the dicalcium phosphate but was yellow in Skellysolve. Pigment 2 was closely associated with pigment 1, in that it was separated from pigment 1 only by continued washing with the solvent. Pigment 2 was green on the column of dicalcium phosphate and yellow in Skellysolve. The transmission curves of 1 and 2 were not always identical for each isolation, probably because of changes taking place on the column during the washing process. Pigment 3 appeared in the largest quantities and assumed a rather broad band on the dicalcium phosphate and calcium carbonate. Pigment 4 washed through the columns of dicalcium phosphate and calcium carbonate rather easily and assumed a rather narrow band. The last two pigments were yellow on the adsorbents and Skellysolve B.

Biological Activity of Noncarotene Chromogens

In order to show whether the results obtained by the proposed chemical method and the biological method are comparable, the vitamin A activity of the noncarotene chromogens removed should be known.

The Peterson-Hughes method of separation was applied to a sample of dehydrated leaf meal. The final extract was washed with water to remove the alcohol and passed through a column of dicalcium phosphate. The noncarotene chromogens were eluted with methyl alcohol, concentrated, and fed to rats, using the Sherman-Todhunter method (*7*), at a rate equivalent to 240 micrograms of carotene. Reference oil was fed at the rate of 30 International units.

Preliminary results indicate that these chromogens have some activity which amounted to 1 to 2 per cent of the total carotene content of the original sample; further studies may alter these figures. At least four pigments appear to be involved with alfalfa meal, but no data are available as to which are responsible for the activity or the quantities of each present at the various stages of change that apparently take place. Therefore the biological evaluation of these chromogens must await further work. However, it is not to be expected that the biological value of these noncarotene chromogens will be large in proportion to the total carotene content of a specific sample.

Stability of Carotene

It was necessary to determine whether the adsorbent changed or removed any carotene while the solution was filtering through the column.

Twelve grams of dicalcium phosphate were placed in each of two columns, and 30 ml. of petroleum ether were drawn through 50 ml. of the carotene solution, followed by 60 ml. of petroleum ether. The carotene value was then determined, using a photoelectric colorimeter. Fresh columns were again made up and the same solution of carotene was filtered again. This procedure was repeated 4 times.

The results are shown in Table III, in which the galvanometer readings at a 1-ml. depth are recorded. Very little carotene was lost, which indicates that the dicalcium phosphate does not alter the chromogen value of the carotene when it is filtered through the column.

Table III. Effect of Filtering Carotene Solution through Column of Dicalcium Phosphate (Lot 102,839)

Times through Column	Galvanometer Readings		
	Solution 1	Solution 2	Control
0	51.8	51.8	51.8
1	52.0	52.0	51.8
2	51.8	51.5	51.5
3	52.0	52.5	51.8
4	52.5	52.8	51.7

Presence of Other Noncarotene Chromogens

To determine whether the dicalcium phosphate removed all the carotenoid pigments other than carotene, the petroleum ether extract of dehydrated leaf meal which was known to contain the noncarotene chromogens was first passed through a column of dicalcium phosphate, and was then subjected to further chromotographic analysis using a column of magnesium oxide. Magnesium oxide had been used by Strain (*8*) to separate the various carotenoids and for their purification. Only one band developed, indicating that only beta-carotene was present in the solution after it passed through the column of dicalcium phosphate.

Cryptoxanthin from yellow corn washed through much more slowly than beta-carotene, so that the method offers a possible way of estimating these two constituents in yellow corn.

Discussion

It is apparent from Table I that the proposed method is in good agreement with the Peterson-Hughes method (*5*) where fresh material is the subject of study. However, with plant material which has been subject to storage or to the digestive tract of a cow, the results are sometimes more than 30 per cent lower.

The interpretation placed on these results, as others have likewise indicated, is that certain carotenoidlike chromogens develop which remain in the petroleum ether phase when it is washed with methyl alcohol. These chromogens are for the most part biologically inactive and should therefore not be included in the final carotene value.

Preliminary biological assay showed that these impurities contained approximately 1 to 2 per cent of the carotene value of the sample. Therefore, when samples are analyzed by this method the results on a biological basis will be low. This error is much smaller than that obtained when using the Willstätter-Stoll method or its modifications, where the error may be as great as 30 per cent.

That these noncarotene chromogens are present in the plant material before it is subjected to analysis and are not the result of treatment during the analytical procedure is demonstrated by the fact that with fresh material there was little difference between the results by the two methods.

Using dicalcium phosphate no special precautions were necessary, at least with the different lots used thus far. No special preparation was required before use, and the column is easy to prepare; the phosphate is merely placed in the column in about two portions with vacuum and light tamping with a plunger.

The transmission curves show that the noncarotene chromogens as isolated are not beta-carotene, although both No. 3 and No. 4 have curves similar to beta-carotene but with different maxima.

In the author's laboratory magnesium oxide and magnesium carbonate have not given good results because they tend to change some of the carotene during the filtration process, thereby giving too low values.

The use of the Tswett column is thought to have some advantages over the method of Fraps where the adsorbent is placed directly in the carotene solution, in that it is possible to see the location of the bands of noncarotene chromogens and know that they have been definitely held by the adsorbent. Further work may also reveal which of the noncarotene chromogens possess biological activity, so that if a Tswett column is used, the column can be removed and the band in question cut out, eluted, and returned to the final extract to be determined as carotene.

It is possible to use alkali for digestion of the sample as in the Peterson-Hughes method in conjunction with the proposed method. It is only necessary to remove the alcohol from the final extract and run the extract through the column.

A word of caution should be given here. One lot of petroleum ether encountered in these studies was contaminated with some substance that permitted all the carotene to go through the column. Washing the petroleum ether with water corrected this difficulty.

Summary

A method for the determination of carotene in plant material consists essentially of extraction with alcohol, extraction of the alcoholic extract with petroleum ether, removal of alcohol from the petroleum ether, and passing the extract through a Tswett column of dicalcium phosphate.

By this method certain noncarotene chromogens are removed, so that the value obtained is probably for pure carotene.

These results indicate that the Willstätter-Stoll method or its modifications for the determination of carotene are not accurate for plant material which has been subjected to storage or to the action of the digestive tract.

Acknowledgment

The author wishes to express his appreciation to C. A. Hoppert of the Chemistry Department for the biological assay of the noncarotene chromogens.

Addendum

Since this paper was prepared for publication one lot of Baker's dicalcium phosphate (21,940) has been encountered which permitted filtration at a slow rate and destroyed some carotene in the process.

A satisfactory phosphate for the determination can be made by dissolving 142 grams of disodium hydrogen phosphate in 1 liter of water and 115 grams of calcium chloride in 750 ml. of water. The calcium chloride solution is poured into the disodium phosphate solution and mixed. The precipitated dicalcium phosphate is placed in a 20-cm. (8-inch) Büchner funnel and washed with three 500-ml. portions of water. The water is drawn off just down to the level of the precipitate, so that it remains wet. The precipitate is transferred to an evaporating dish and dried for 24 hours at 100° C. The dried dicalcium phosphate is then broken up in a mortar, after which it is ready for use. It is important that the phosphate be left wet and dried in an evaporating dish.

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Determination of Glycerol

By Oxidation with Ceric Sulfate in Fermentation Media Containing Dextrose

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IN STUDIES on the production of glycerol by fermentation a major difficulty was the lack of a rapid and accurate method for the quantitative determination of glycerol in the presence of sugar.

Procedures proposed for the determination of glycerol in the absence of sugar usually involve either oxidation by dichromate or modifications of the method of Benedikt and Cantor (*2*). A procedure of Hoyt and Pemberton (*8*) consists of a sugar determination by the Munson and Walker (*9, 15*) method, followed by oxidation of the glycerol-sugar mixture with a sulfuric acid–dichromate mixture. The method requires a large excess of dichromate and 3 hours of heating. Objections to the acetin method have been summarized by Andrews (*1*), while appraisals of the dichromate and other procedures have been made by Hoyt and Pemberton (*8*). The acetin method is most applicable in concentrated glycerol solutions (above 50 per cent), but even when the procedure is used as modified by Shaefer (*10*) the sugar is susceptible to acetylation and thus would interfere with the analysis of glycerol. Determination of glycerol by means of ceric sulfate was investigated by Cuthill and Atkins (*4*). The analysis was attempted only in the absence of sugar.

The present communication gives details of a simple and rapid method for the determination of glycerol, in the presence of dextrose, which is applicable to a fermented medium. The procedure involves the determination of the sugar by a copper titration method, followed by the oxidation of another sample with ceric sulfate under standardized conditions. Both the sugar and the glycerol are oxidized, but from a correction for the ceric sulfate used by the sugar, the glycerol is calculated by means of suitable equations or read from a graph.

Experimental

Several procedures were tested for the determination of the sugar, including the Shaffer-Hartmann (*11*) method, the Hagedorn-Jenson (*7*) ferricyanide method as modified by Blish and Sandstedt (*3*), and the Shaffer-Somogyi (*12, 14*) copper method. The latter procedure, employing an alkaline copper reagent followed by an iodometric titration of the reduced copper, proved to be the most suitable and specific under the conditions employed. The procedure was modified slightly to obtain more reproducible and accurate results. The modifications involved heating appropriately diluted protein-free samples, mixed with a fixed amount of the reagent, in 22 × 250 mm. Pyrex test tubes in a water bath for 35 minutes instead of the 20 minutes recommended by the authors. The tubes were covered with glass bulbs during the heating and until titration to prevent the entry of oxygen.

In Table I are presented data on the analysis of mixtures of dextrose and glycerol with 0.1000 *N* ceric sulfate solution; the details of the procedure are given below. The standard solutions of dextrose and glycerol contained 2.000 mg. of pure reagent per ml. of solution; freshly prepared solutions were employed in each case to obviate any errors which might result from the possible growth of microorganisms.

The sample is diluted so as to contain between 0.030 and 0.500 mg. of dextrose per ml. and the exact quantity is determined by the modified Shaffer-Somogyi procedure given above. For the ceric sulfate oxidation a sample is diluted so that the total

Table I. Analysis of Mixtures of Glycerol and Dextrose by Oxidation with 0.100 *N* Ceric Sulfate

Glycerol			Dextrose		
Present	Found	Error	Present	Found	Error
	Mg. per ml.			*Mg. per ml.*	
0	..	....	0.50	0.52	+0.02
0	..	....	1.00	1.00	±0.00
0	..	....	1.50	1.53	+0.03
0	..	....	2.00	1.99	−0.01
0	..	....	2.50	2.50	±0.00
0	..	....	3.00	2.99	−0.01
0	..	....	4.00	3.96	−0.04
0	..	....	5.00	4.80	−0.20
0.47	0.47	±0.00	0	..	....
0.95	0.94	−0.01	0	..	....
1.00	1.01	+0.01	0	..	....
1.41	1.42	+0.01	0	..	....
1.89	1.89	±0.00	0	..	....
2.00	2.02	+0.02	0	..	....
2.36	2.37	+0.01	0	..	....
2.83	2.87	+0.04	0	..	....
3.00	3.01	+0.01	0	..	....
3.78	3.76	−0.02	0	..	....
4.00	4.03	+0.03	0	..	....
5.00	4.93	−0.07	0	..	....
0.50	0.49	−0.01	0.50	..	....
1.00	1.00	±0.00	1.00	..	....
1.00	1.01	+0.01	2.00	..	....
1.00	1.00	±0.00	3.00	..	....
2.00	1.99	−0.01	1.00	..	....
2.00	2.02	+0.02	2.00	..	....
2.50	2.51	+0.01	2.50	..	....
3.00	2.98	−0.02	1.00	..	....

TABLE II. ANALYSIS FOR GLYCEROL IN FERMENTATION MEDIUM BY OXIDATION WITH 0.100 *N* CERIC SULFATE

Dextrose in Sample	Glycerol in Sample	Dextrose Added	Glycerol Added	Total Glycerol Found	Added Glycerol Found	Error
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.07	1.63	0	0	1.63	..	....
0.07	1.63	0	0.50	2.14	0.51	+0.01
0.07	1.63	0	1.00	2.63	1.00	±0.00
0.07	1.63	0	1.50	3.13	1.50	±0.00
0.07	1.63	0.50	0.50	2.13	0.50	±0.00
0.07	1.63	0.50	1.00	2.61	0.98	−0.02
0.07	1.63	1.00	0.50	2.14	0.51	+0.01

quantity of dextrose plus glycerol does not exceed 4 mg. per ml.; best results are obtained between 0.50 and 2.50 mg. Into each 22 × 175 mm. Pyrex test tube are measured exactly 5 ml. of sample, approximately 2 ml. of 1 to 1 sulfuric acid, and 5.00 ml. of 0.1000 *N* ceric sulfate in 1 molar sulfuric acid. The contents of the tubes are then well mixed. A sample of less than 5 ml. may be used, providing water is added to bring the total volume to 12 ml. The tubes are then heated in a boiling water bath for 1 hour. The tubes are covered with glass bulbs during the heating to prevent excessive evaporation with subsequent crystallization of salts. More consistent results were obtained with the covered than with the uncovered tubes, although the general mathematical relation between concentration and ceric sulfate consumed is the parabolic function discussed below.

At the end of the period of heating, the tubes are placed in cold water and cooled to 20° to 25° C. The excess ceric sulfate is titrated with 0.1000 *N* ferrous ammonium sulfate solution which is kept under an oxygen-free atmosphere. An excellent end point is obtained with either of the oxidation-reduction indicators, erioglaucin or the *o*-phenanthroline ferrous complex of Smith (*13*). Variations in sulfuric acid from 1 to 5 ml. had no significant effect. The final titration of the ceric sulfate may be made within 2 hours after cooling, although immediate titration is recommended.

An examination of the data for pure dextrose and pure glycerol shows the volume of ceric sulfate used to be a parabolic function of the concentration of the reagent. The calculations are made as follows:

The volume in milliliters of 0.1000 *N* ceric sulfate, V_1, used by the weight of dextrose in milligrams, D, determined by analysis is calculated by Equation 1.

$$\log (V_1 \times 10) = 1.091 \log (D \times 10) - 0.307 \qquad (1)$$

By subtracting V_1 from the total volume of ceric sulfate used, V, the volume of ceric sulfate consumed by the glycerol, V_2, is obtained. The amount of glycerol (mg.) is obtained by the relation of Equation 2.

$$\log (G \times 10) = 1.000 \log (V_2 \times 10) + 0.069 \qquad (2)$$

The above equation simplifies to

$$G = 1.172\ V_2 \qquad (3)$$

In the corresponding equations for the uncovered tubes the constants differ slightly, so that

$$\log (V_1 \times 10) = 1.085 \log (D \times 10) - 0.327 \qquad (1a)$$

$$\log (G \times 10) = 1.027 \log (V_2 \times 10) + 0.063 \qquad (2a)$$

Calculations for glycerol in mixtures of glycerol and dextrose by means of Equations 1 and 2 are given in Table I. The procedure gives entirely satisfactory results for mixtures of the pure reagents.

In Table II are given data for media which had been subjected to alcoholic fermentation. The medium contained dextrose, yeast extract (Difco), ammonium chloride, calcium chloride, magnesium sulfate, secondary potassium phosphate, and tap water. After completion of the yeast fermentation, the alcohol was distilled. An aliquot of the residual liquid was clarified with basic lead acetate and then subjected to the analytical procedure given above. Analyses were made of the clarified samples and also of the samples to which known amounts of glycerol and dextrose had been added. The data show the procedure to be satisfactorily applicable to the determination of glycerol in the presence of dextrose in a fermentation medium.

The analysis of glycerol in a fermentation medium, containing dextrose as substrate, may be accomplished by the methods given in the present communication. The fermented medium contains, principally, ethanol together with varying amounts of glycerol, acetic acid, succinic acid, and perhaps some acetaldehyde. Distillation in the presence of calcium carbonate removes the volatile nonacid compounds. The proteins in the residual liquid are removed by precipitation with basic lead acetate. According to Willard and Young (*16*), acetic and succinic acid do not interfere in analysis by means of ceric sulfate. Tartaric and malonic acids will interfere since they may be determined quantitatively with ceric sulfate; however, the latter compounds would not be expected in the fermentation. Sodium and potassium salts in appreciable quantities interfere because of the formation of slightly soluble complex salts with ceric sulfate in the presence of sulfuric acid. However, the above salts are not usually present in fermentation media in sufficient concentrations to cause difficulty, especially since the sample must be diluted so that the combined weight of the glycerol and sugar does not exceed 4 mg. per ml.—i. e., 0.4 gram per 100 ml. Lithium and ammonium salts do not interfere with the determination. The latter is especially important, since some of the work in progress in our laboratories on the glycerol fermentation deals with the regulation of pH by the use of ammonium hydroxide, ammonium carbonate, and other ammonium salts. Ammonium sulfite is also being employed in place of sodium sulfite. The ammonium compounds are being used principally because of their relative ease of removal at the end of the fermentation in order to decrease the difficulty of recovery of the glycerol after the fermentation. These studies are being made at constant pH by means of a control adaptation of the Cameron pH recorder.

Reproducible results have been obtained in this laboratory for analyses made at different times and with different sets of reagents. Nevertheless, it would be advisable for the individual analyst to standardize his methods and technique, and to prepare his own parabolic equations from several analytical series for sugar and for glycerol, using reagents of known purity. Since two samples of commercial ceric sulfate may not be identical in composition, slight differences in equation constants may be noted. The variation of the constants is especially noticeable if one uses ceric sulfate solutions which are not of identical normality.

The analytical method described should be adaptable to many other types of analyses where an economical, rapid, and accurate method is desired, such as analyses of mixtures of polyhydric alcohols and reducing sugars of various types. This type of procedure would be much simpler than the periodate methods of Fleury and Fatome (*5*, *6*).

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Photometric Determination of Potassium with Dipicrylamine

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KOLTHOFF and Bendix (*1*) describe the use of dipicrylamine in the gravimetric, volumetric, and colorimetric determination of potassium. Several problems were encountered by the author in the adaptation of the colorimetric procedure to the determination of potassium in tissues and blood. It was found that information regarding the selection of a light filter for this determination was not available, and that modification of the procedure to reduce the amount of necessary manipulation and to allow working at room temperature was desirable. A revision of the procedure of Kolthoff and Bendix and a new indirect method are described below.

Selection of Light Filter

In the colorimetric procedure of Kolthoff and Bendix, and in the work described below, the concentrations of known solutions of potassium dipicrylaminate are plotted against the light transmission percentage ($I/I_0 \times 100$) and unknown samples are compared with the curve obtained. A group of such curves, using different light filters, is shown in Figure 2. The sensitivity of the determination to changes in the potassium dipicrylaminate concentration varies greatly with the light filter used and the thickness of solution through which the light passes. The latter factor can be rendered unimportant by using identical cells to contain solutions to be subjected to transmission determination. The effect of a light filter in increasing the sensitivity is best determined by experiment, although examination of the transmission curves of various concentrations of potassium dipicrylaminate will be helpful in a preliminary selection of filters.

A Cenco-Sheard-Sanford photelometer was used for measuring transmission percentages. In this instrument, the light passes horizontally through a light filter, then through a cell having parallel glass walls 1 cm. apart, and finally falls on a photoelectric cell which actuates a microammeter. A slide allows any of the three identical cells to be placed between the filter and the photoelectric cell. One cell is filled with the pure solvent, and this acts as the standard with a transmission percentage of 100. The transmission percentages of the other solutions are determined by adjusting the light source so that the instrument reads 100 with the standard cell in place, and then substituting the cell containing the solution of unknown transmittancy.

With a green glass filter (supplied with the photelometer for use in hemoglobin determinations) this instrument proved to be insensitive, when compared to the results obtained by Kolthoff and Bendix with a Lange colorimeter. Following their suggestion, blue filters were substituted for the green filter. Cobalt blue glass and blue cellophane were tried, but did not give improved results.

As the first step in finding a better light filter, the absorption spectra of several solutions of potassium dipicrylaminate were determined with the Hilger visual spectrophotometer. Figure 1 shows three of these curves. While they are rough, it is apparent that the filter should have a sharp cutoff at about 5300 Å., and allow as little as possible of the red or infrared to come through. The cobalt glass and the blue cellophane failed because both have substantial transparency to red light, whereas the absorption of solutions of potassium dipicrylaminate of widely differing concentrations is almost the same.

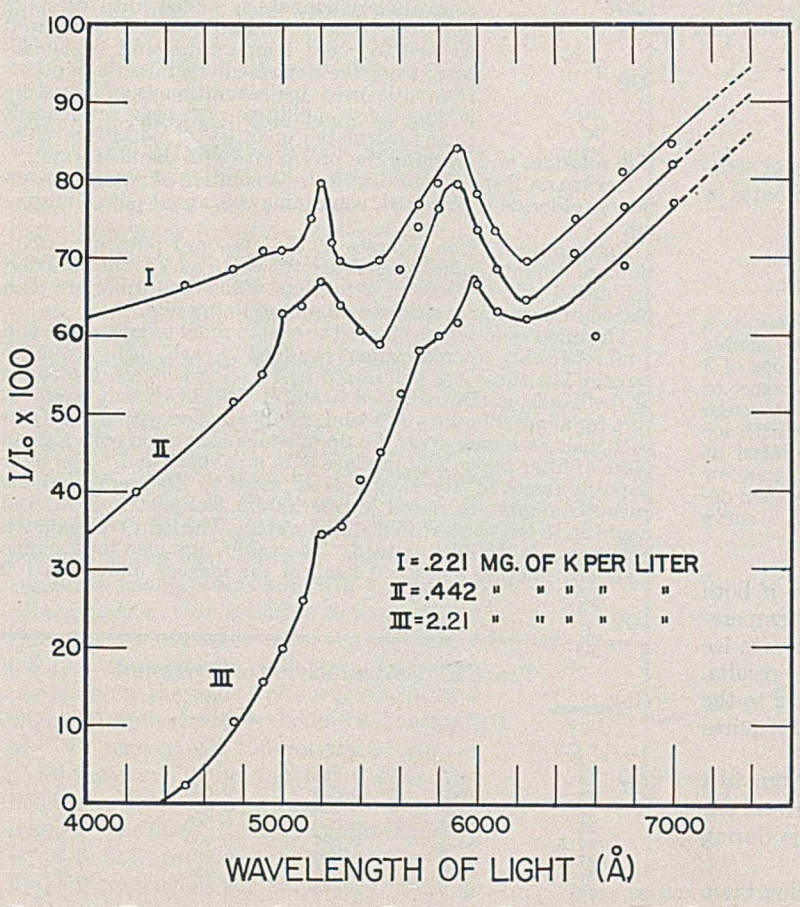

FIGURE 1. TRANSMISSION CURVES OF POTASSIUM DIPICRYLAMINATE SOLUTIONS

At the suggestion of the Corning Glass Works, filters of Corning glasses No. 556 (signal blue) and No. 429 (blue green) were obtained. In Figure 2 are plotted the logarithms and the values of the transmission percentages of dipicrylaminate solutions as a function of the concentration, using several different light filters. The values of Kolthoff and Bendix are not strictly comparable to the others, as the solution thickness in the cells used by them was much greater than the 1 cm. of the photelometer cells. Several important conclusions may be drawn from the curves. The equipment used by Kolthoff and Bendix gives superior results up to a concentration of 50 micrograms of potassium as potassium dipicrylaminate per 100 ml. The use of a 1-cm. cell and a combination of Corning glasses Nos. 556 and 429 will give better results over a larger range of concentrations. If a single filter must be used, No. 556 is preferable.

It can be seen from curve I (Figure 2) that dipicrylamine solutions follow Beer's law closely if the combination of filters is used. This makes possible the use of a Duboscq colorimeter if a photoelectric instrument is not available. Care must be

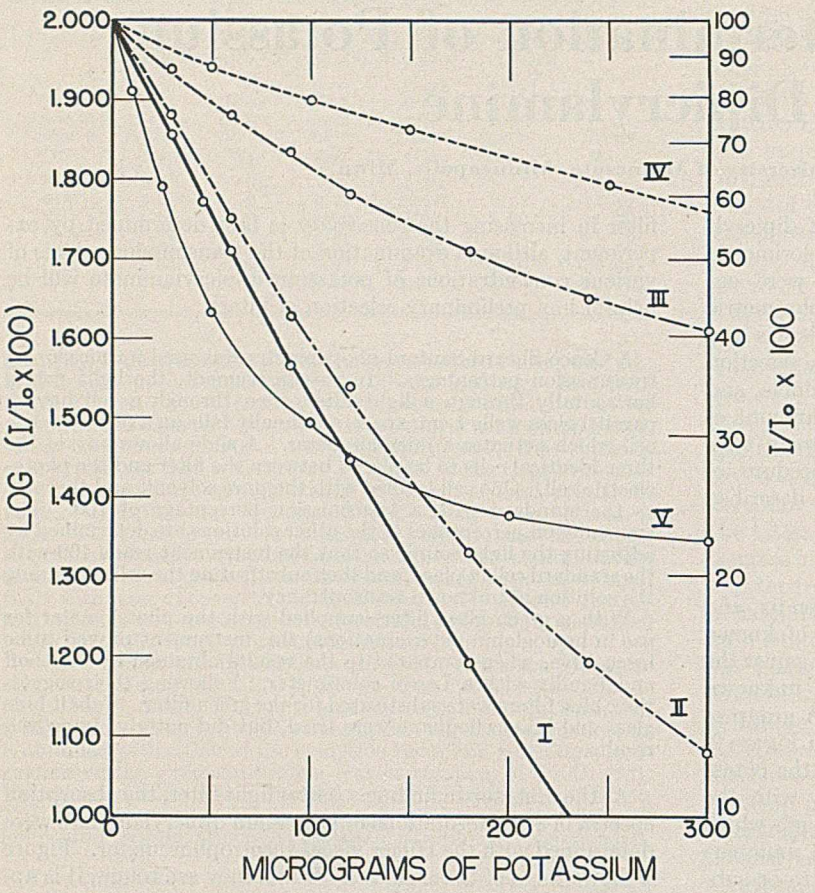

FIGURE 2. TRANSMISSION PERCENTAGE PLOTTED AS A FUNCTION OF CONCENTRATION OF POTASSIUM DIPICRYLAMINE FOR VARIOUS COMBINATIONS OF LIGHT FILTERS

I. 5.5 mm. of Corning glass No. 556 and 3.8 mm. of Corning glass No. 429
II. 5.5 mm. of Corning glass No. 556
III. 3.8 mm. of Corning glass No. 429
IV. Green glass light filter
V. Values of Kolthoff and Bendix

taken, however, that the concentration and thickness of solutions fall within the range where curve I approximates a straight line.

Revision of Kolthoff-Bendix Procedure

In their colorimetric method, Kolthoff and Bendix evaporate a liquid to dryness in a porcelain crucible, place the cooled crucible in ice water to a depth of 1 cm., and then add a few drops of 3 per cent magnesium dipicrylaminate. They allow the mixture to stand for 10 minutes, and then draw off the excess reagent through a filter stick. They wash the precipitate successively with ice water, a solution of potassium dipicrylaminate (saturated at 0° C.), and finally with ice water, dissolve the precipitate in a few drops of acetone, and dilute with water to 100 ml. They then determine the transmission percentage, and compare with a curve such as those of Figure 2.

This procedure may be used at room temperature if both the reagent and the wash liquid are saturated at that temperature with potassium dipicrylaminate. The washings with ice water are not necessary in order to get reproducible results, as the amount of potassium salt in the liquid adhering to the precipitate and apparatus is negligible and at the most requires a slight constant blank correction.

It is also advantageous to run the whole determination in a large Pyrex test tube calibrated at 50 ml., and to perform the final dilution in it, thus eliminating the chance of loss during the transfer from a crucible to a volumetric flask.

Three 100-microgram samples were determined using these modifications with errors of 0, 0, and +1 per cent. Two 50-microgram samples gave results of +1 and +2 per cent, respectively.

Indirect Procedure for Potassium

An indirect empirical method for potassium was developed which has certain advantages over the procedures described above. It is more rapid, requires less manipulation and apparatus, uses less of the reagent per determination, and gives more accurate results.

The reagent used is a 0.6 per cent solution of lithium dipicrylaminate in water, saturated at 25° C. with potassium dipicrylaminate. A measured quantity of this reagent is added to the dried sample, and the mixture is allowed to stand for 2 hours. An aliquot portion of the supernatant liquid is then withdrawn and diluted to 100 ml., and the transmission percentage is determined. The value obtained is compared with a standard curve obtained by treating a graduated series of standard samples in the same manner. A standard curve of this type is shown in Figure 3.

MATERIALS USED. Dipicrylamine is obtainable from the Eastman Kodak Company at $2 per 10 grams.

APPARATUS. If the laboratory is subject to great temperature variation, a crude thermostat controllable to ±2° C. is required.

LITHIUM DIPICRYLAMINATE REAGENT, 0.6 per cent. A solution of 0.55 gram of lithium carbonate in 100 ml. of water is prepared, and 3 grams of dipicrylamine are added. This solution is heated to 50° C., allowed to stand for 24 hours, then filtered into a 500-ml. flask and made up to about 500 ml. This diluted reagent is heated to 50° C. and moist potassium dipicrylaminate is added until no more dissolves. The saturated solution is allowed to cool to room temperature and is not filtered from the deposited crystals. The potassium salt used for saturation is prepared by adding a few milliliters of 3 per cent reagent to the calculated quantity of potassium chloride solution, and washing the precipitate with distilled water.

STANDARD POTASSIUM SOLUTION. A solution of potassium sulfate or chloride is prepared, containing 0.1 mg. of potassium per ml.

PROCEDURE. Nine samples of the standard potassium solution are run into conical centrifuge tubes (15 ml.) so that the first contains 1 ml., the second, 2 ml., etc. The nine tubes are then placed in a drying oven and evaporated to dryness.

The dried samples are allowed to cool to room temperature, and 1 ml. of freshly filtered reagent is added to each, using the same pipet. The tubes are well mixed by rotating between the palms of the hands, and are allowed to stand at a convenient temperature for about 2 hours. A 0.4-ml. sample is then withdrawn from each, using a blood pipet, the tip of which is covered with a small piece of filter paper held in place with a rubber band. The filter paper is removed, the sample is adjusted to the mark, and the entire contents are rinsed into a 100-ml. volumetric flask, and made up to the mark with distilled water. The light transmission percentage is then determined. The values obtained for the nine standard samples and for 0.4 ml. of the reagent diluted to 100

TABLE I. DETERMINATION OF POTASSIUM

Temperature	$I/I_0 \times 100$	K Found	K in Sample	Error
° C.		Mg.	Mg.	%
18	58.5	0.621	0.600	3.5
18	59.0	0.624	0.600	4.0
22	55.0	0.606	0.600	1.0
25	54.0	0.600	0.600	0.0
27.5	53.5	0.597	0.600	−0.5
27.5	54.0	0.600	0.600	0.0
34	48.5	0.570	0.600	−5.0
34	48.5	0.570	0.600	−5.0

TABLE II. DETERMINATION OF POTASSIUM

Time of Standing	$I/I_0 \times 100$	K Found	K in Sample	Error
Min.		*Mg.*	*Mg.*	%
2	44.0	0.540	0.600	−10
5	48.0	0.565	0.600	− 5.8
15	51.5	0.587	0.600	− 2.2
60	53.5	0.597	0.600	− 0.5
125	54.0	0.600	0.600	0.0
180	54.0	0.600	0.600	0.0
24 hours	54.0	0.600	0.600	0.0

TABLE III. DETERMINATION IN THE PRESENCE OF SODIUM

K in Sample	$I/I_0 \times 100$	K Found	Error
Mg.		*Mg.*	%
0.000	7.5	0.050	
0.100	9.5	0.130	+30.0
0.200	13.0	0.210	+ 5.0
0.300	19.0	0.310	+ 3.3
0.400	27.0	0.405	+ 1.25
0.500	37.5	0.505	+ 1.0
0.600	55.5	0.610	+ 1.6
0.700	72.5	0.710	+ 1.2
0.800	84.0	0.790	− 1.1
0.900	90.0	0.865	− 4.0

ml. are plotted to give a standard curve, such as the curve of Figure 3.

Unknowns are determined by treatment in the same manner, using the same pipets used in preparing the standard curve, and comparing the observed transmission percentage with it.

Examination of the plot of Figure 3 will show that the most exact determinations may be made with samples containing from 0.3 to 0.8 mg. in the quantity taken for analysis. However, the photelometer used in this laboratory proved to give results reproducible to 0.1 scale division in the lower readings, and to only 0.5 division in the readings near 100 per cent transmission. (One division corresponds to 1 per cent of transmitted light.) Thus, samples of 0.15 mg. may be read with a reproducibility of ±1.3 per cent, and the optical error of determining larger samples is negligible. The reproducibility of the results using this procedure thus seems to depend almost entirely on the reproducibility with which the chemical procedure may be carried out. It is essential, therefore, that for highest accuracy the same pipets should be used for both the standards and the unknowns, and in the case of the 1-ml. delivery pipet, a reproducible drainage procedure is also essential.

Samples containing less than 0.15 mg. of potassium in the amount taken for analysis may be determined by plotting a curve with a more dilute reagent, or adding 0.5 mg. of potassium to each sample. Of these two alternative methods, the second is the more desirable, since the use of a more dilute reagent will lengthen the time necessary for complete precipitation and will render the determination more sensitive to temperature changes. On the other hand, the addition of 0.5 mg. of potassium will place the determination in the region of curve of Figure 3 where the optical error is about ±0.2 per cent, or ±1.2 per cent of a 100-microgram sample.

In the work done by the author, the lithium salt of dipicrylamine was used as a reagent because in some determinations large quantities of magnesium or sodium may be present in the sample or may be introduced during the preparation of the sample, and the use of a magnesium or sodium reagent might act to increase the concentration of the particular ion to the point where appreciable coprecipitation takes place.

Because of the variation of the solubility product of the potassium salt with temperature, analyses should be run at about the same temperature at which the standard curve was determined. Table I shows a series of determinations run in the same way, except that they were allowed to stand at different temperatures. It can be seen that the determination should be run within ±3 per cent of the temperature at which the standard was determined.

Table II shows a similar series of determinations conducted at 25° C., using the procedure given above, but varying the time of precipitation. Equilibrium is established in 2 hours or less. Experiments conducted in the same manner, using a 0.25 per cent reagent at 28.5° C., gave values which did not become constant for over 4 hours.

Determination of Potassium in Presence of Sodium

Since the determination of potassium in the presence of large amounts of sodium is important, some determinations were made to find out the effect of this ion. A series of standard potassium samples was prepared, according to the directions given in the procedure. To each of these, 1 ml. of physiological saline (about 3.5 mg. of sodium per ml.) was added before evaporation. The results of the subsequent analyses are shown in Table III.

Table III indicates that the curve of Figure 3 may be used with samples containing between 0.4 and 0.8 mg. of potassium and a sodium concentration of about 3.5 mg. per ml. if a correction is applied. A better plan is available, however. The transmission percentages of Table III may be plotted to give a

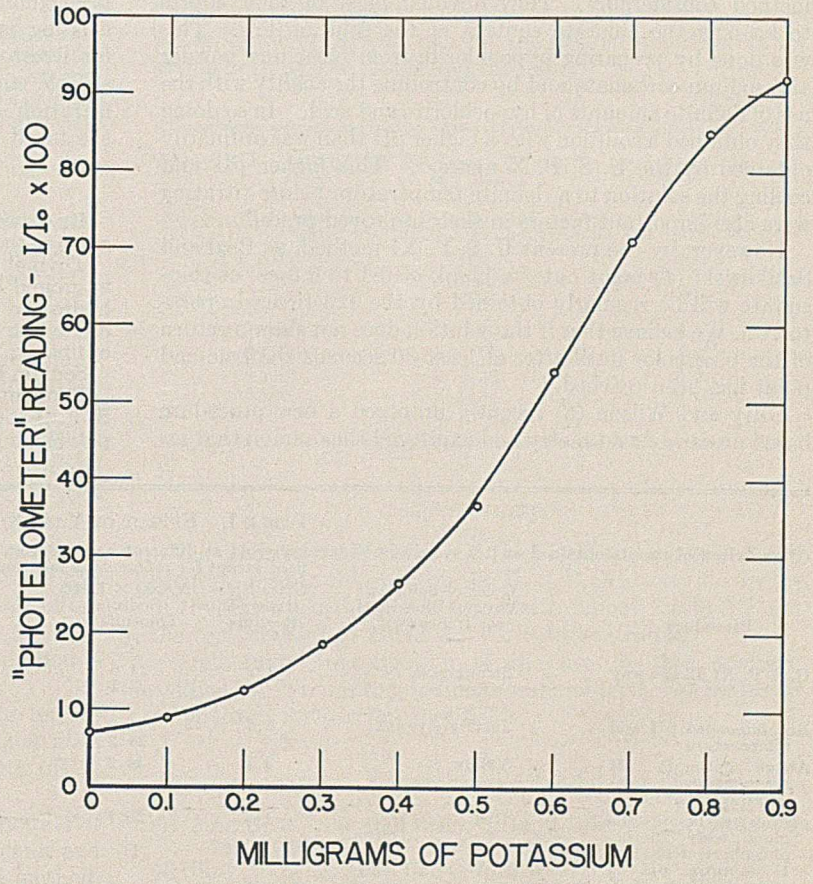

FIGURE 3. TYPICAL CALIBRATION CURVE FOR INDIRECT PROCEDURE FOR POTASSIUM

standard curve which may be used for the determination of a wider range of concentrations. The concentration of sodium should be relatively constant, but variations of ±10 per cent or so in the concentration will have little effect on the accuracy of the determination. An example of the use of such a curve is in the determination of potassium in blood plasma, in which the sodium concentration seldom varies more than 10 per cent from the normal values.

Application to Larger Quantities

The method outlined above can be used for the determination of larger quantities of potassium. Quantities as large as 80 mg. may be determined by using larger quantities of the reagent, and taking 0.4 ml. of the supernatant liquid for transmission analysis. The resulting transmission percentage can be compared with the standard curve, and the result multiplied by the number of milliliters of reagent used.

Acknowledgment

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Iodine in Thyroid

Elimination of Uncertain End Point and Blank in the U. S. P. XI Assay

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IN COMMON with other laboratories the authors had difficulty several years ago obtaining consistent results in determining iodine in thyroid by the old U. S. P. X method. At that time the senior author found that simple pH adjustment of the solution to be titrated eliminated the indefinite end point and no blank was obtained on the reagents. After the U. S. P. XI method was available, this refinement greatly improved its accuracy and precision also.

The work of Beal and Szalkowski (*1*), upon which the present U. S. P. XI method is based, improved upon the older method considerably. They devoted most of their efforts to keeping the chlorate content of the solution low. This was done by preparing hypochlorite from bleaching powder and sodium carbonate and by controlling the acidity with the use of definite amounts of hypochlorite and acid. In so doing they obtained a solution with a higher pH than was ordinarily obtained by the U. S. P. X method. This higher pH and cooling the solution to a definite temperature before titrating were also important factors in their improved procedure.

However, by the present U. S. P. XI method, as Beal and Szalkowski (*1*) point out, "a blank of 0.4 to 0.6 cc. of thiosulfate will be regularly obtained by the experienced operator. . . . We believe that if the solution does not show a return of the blue color until after at least 30 seconds the true end point has been reached."

Hilty and Wilson (*3*) recently proposed a new procedure based on cerate oxidimetry and expressed the opinion that inconsistent results obtained by the present U. S. P. XI method are due to technical difficulties in eliminating excess chlorine.

Recent difficulties in checking with other laboratories and their eventual ability to check the authors' results when the suggested modification was used lead the authors to believe that a note should be published regarding this refinement. If the uncertain end point and blank can be eliminated, there is an advantage in retaining the present method because of the recognized sharpness of the starch-iodine end point.

This modified U. S. P. XI thyroid method has recently been found more accurate and less time-consuming than existing procedures for determining iodine in mineral feeds for livestock. It is, however, necessary to use a 5.0-gram sample and to remove the insoluble material present by filtration after the soluble material has been dissolved from the fused mineral feed sample.

Modified Method

REAGENTS. These reagents are required in addition to those given in the U. S. P. XI assay for iodine in thyroid.

Thymol blue, 0.4 per cent. One gram of thymol blue powder is ground in an agate mortar with 21.5 cc. of 0.1 *N* sodium hydroxide plus enough water to dissolve the dye and is finally diluted to 250 cc. This is ten times the concentration of the ordinary Clark and Lubs indicator solutions.

Sodium hydroxide, approximately 50 per cent solution.

PROCEDURE. The procedure is the same as the present U. S. P. XI assay for iodine in thyroid up to the addition of the potassium iodide. Before it is added, the pH of the solution is

TABLE I. EFFECT OF VARYING pH

(Comparison of results obtained on 1.0 gram of powdered thyroid at different hydrogen-ion concentrations of the solution titrated. U. S. P. XI method used except for refinements mentioned.)

Procedure	Color of Solution (Thymol Blue Used for Indicator)	Glass Electrode pH Reading	*N*/200 × 1.046 Sodium Thiosulfate	Blank	Iodine	Remarks
			Cc.	*Cc.*	%	
U. S. P. XI all the way	Strong pink	2.2	24.6 25.1	0.2 0.2	0.270 0.275	Blue starch-iodine color returned in 1 to 2 minutes
Recommended pH and temperature	Just barely pink	2.6	24.2 24.2	None None	0.265 0.265	No return of blue color at end of 30 minutes
Above optimum pH recommended, temperature of 33° C.	Yellow	3.1	24.2	None	0.265	Reaction slow and 10 minutes required to reach stable end point
pH lowered below optimum with 20 cc. phosphoric acid (1 to 1) (approx. pH as with old U. S. P. X method)	Red	1.5	25.7	Indefinite	0.284	Color returned in a few seconds and after 5 minutes an additional 1.0 cc. of thiosulfate solution over the 25.7 was required

TABLE II. ACCURACY OF METHOD

(Potassium iodide equivalent to 2.0 mg. of iodine used, instead of powdered thyroid, at different hydrogen-ion concentrations of solution titrated. U. S. P. XI method except for refinements mentioned.)

Procedure	Color of Solution (Thymol Blue Used for Indicator)	$N/200 \times 1.046$ Sodium Thiosulfate	Blank	Iodine	Recovery
		Cc.	*Cc.*	*Mg.*	%
U. S. P. XI all the way	Strong pink	18.4	0.2	2.014	100.7
		18.7	0.2	2.047	102.35
Recommended pH and temperature	Just barely pink	18.0	None	1.992	99.6
		18.0	None	1.992	
pH lowered below optimum with 20 cc. of phosphoric acid (1 to 1)	Red	19.3	Indefinite	2.136	106.8
		18.9		2.092	104.6

adjusted by adding 5 drops of the thymol blue indicator solution and then 50 per cent sodium hydroxide until the solution is barely on the pink side. This corresponds to the T. B. color of pH 2.2 to 2.4 on the Clark color chart and can best be described as a salmon pink. The actual pH measured with a glass electrode is about 2.6. The discrepancy between electrometric and colorimetric readings is, of course, due to a colorimetric salt error. Any uncertainty as to this color the first few times the test is made can be avoided by making the solution yellow and bringing back just to salmon pink with phosphoric acid (1 to 1). The temperature of the solution is adjusted to 32° to 34° C., the potassium iodide is added, and the titration is made with thiosulfate solution in the usual manner.

The pink color of the indicator does not interfere with the iodine-starch end point. If any find its presence objectionable, they can use the thymol blue as an outside indicator. It is also likely that thymol blue test papers could be successfully employed.

The U. S. P. method calls for the titration to be made at about 25° C. When alkali is added to raise the pH, the 25° C. of the U. S. P. method rises to about 33° C. and best results are obtained at this higher temperature. If a higher temperature is not used at the higher pH, the liberation of iodine proceeds slowly when the potassium iodide is added.

The hypochlorite made according to the older U. S. P. X and hypochlorite made in the packing-house sanitary department from commercial chemicals all give satisfactory results when the method is modified by pH adjustment. Others may have convenient sources of hypochlorite which, when adjusted to a chlorine content of about 2.5 per cent will make its special preparation unnecessary.

Experimental

The experimental work presented is representative of considerable data accumulated on this subject over a period of years. In all cases the results have consistently pointed in the same direction. An analyst will invariably check himself within 0.1 cc. and two analysts will not ordinarily differ more than 0.2 cc. (0.1 per cent) by the modified procedure.

In Table I is shown the effect of varying the pH of the solution to be titrated from the low pH encountered in the old U. S. P. X method up to a pH above the optimum. Electrometric pH readings were made for comparison with the colors of the various solutions. The present U. S. P. procedure gives solutions which vary in pH from 2.0 to 2.4 even with careful measurements of the same set of reagents. The electrometric pH readings of the adjusted solutions will vary between 2.5 and 2.7. The figure 2.6 given is therefore an average pH. The table is self-explanatory.

Table II shows that the accuracy of the refined method is good.

Table III shows that the absence of a blank at the recommended pH is not due to lack of sensitivity to small quantities of iodine. Recovery was good up to and including a pH of 3.0 and with only 0.2 cc. of 0.005 *N* iodate.

Using the recommended procedure, significant amounts of iodine in the reagents used would appear in the blank and would have meaning; whereas, by the U. S. P. XI method, blanks on the same reagents are apt to be variable and therefore of doubtful value.

Likely Cause of Uncertain End Point

The fact that the authors obtain no blank by their method would seem to eliminate the possibility that residual chlorine is at fault.

Hojer (*4*) claims that the errors, when chlorine is used to oxidize iodides to iodates, are due to residual chlorates and perchlorates, and that slight effects can be caused by the oxygen of the air in acid solution. While Hojer realized that the effect of the oxygen in the air could be minimized by the control of acidity, he did not realize that the interference of chlorates could be avoided if the pH was high enough. He finally resorted to the use of bromine for his oxidations.

Bray (*2*) found that within certain limits the rate at which iodine is liberated in solutions containing potassium chlorate, potassium iodide, and hydrochloric acid is proportional to the concentration of the chlorate and to the square of the concentration of the hydrogen ion, and is a linear function of the concentration of the chloride ion and of potassium iodide.

It appears, therefore, that the liberation of iodine from iodides by chlorates at the pH of the U. S. P. titration is slow and incomplete but is sufficient to make the end point uncertain. This effect is increased by lowering the pH or increasing the chlorate content of the solution. When the pH of the solution is raised to about 2.6, the reaction proceeds so slowly that the amount of chlorate ordinarily present does not interfere and no blank is obtained on the reagents. This pH is, however, low enough for the desired quantitative reaction between the iodide and the iodate.

TABLE III. RECOVERY OF SMALL AMOUNTS OF 0.005 *N* IODATE SOLUTION

[In 150 cc. of water adjusted to various pH values with phosphoric acid (1 to 1) and titrated with 0.005 *N* sodium thiosulfate at 32° to 34° C.]

pH of Solution (Glass Electrode)	0.005 *N* Potassium Biiodate	0.005 *N* Sodium Thiosulfate
	Cc.	*Cc.*
2.98	0.50	0.50
2.70	0.50	0.50
2.64	0.50	0.50
2.38	0.50	0.50
3.00	0.30	0.30
2.82	0.20	0.20

Summary

The uncertain end point and the blank are eliminated and accurate results obtained by the U. S. P. XI assay for iodine in thyroid when the pH is adjusted to about 2.5 to 2.7 and the temperature to about 33° C. before titration.

Preliminary investigation of the use of this modified method for determining iodine in mineral feeds has indicated possibilities for use in other fields.

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Electrolytic Determination of Copper in Steel

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AS EARLY as 1910 Blasdale and Cruess (*2*) remarked that the electrolytic determination of copper had been studied since 1864 and that the literature on the method was voluminous. The literature has been greatly increased since that time and a comprehensive review would be a major report in itself. Numerous publications have dealt with the electrolytic separation of copper from iron-bearing solutions, but few have had to do with the actual concentration proportions met in the analysis of copper-bearing steels and cast iron. Present practice appears to be based upon the separation of copper as the sulfide, followed either by its oxidation and weighing as cupric oxide or resolution of the cupric sulfide and electrolysis of the resultant iron-free solution.

The presence of ferric ion during the electrolytic deposition of copper is undesirable (*6*), inasmuch as there is a strong tendency for the iron to be reduced to the ferrous state and thus either prevent the deposition of copper or retard it to such an extent as to make the separation impracticable. Removal of the iron by neutralization with ammonia and filtration, as recommended by Schong (*9*), followed by electrolysis of the acidified filtrate, is a tedious procedure at best. This is especially true in the analysis of low-copper samples, since Toporescu (*12*) has shown that copper is occluded to an appreciable extent under these conditions, thus making double precipitation necessary. The same remarks apply to the method suggested by Fife and Torrance (*5*).

The preferential solubility of iron by dilute sulfuric acid has been suggested by Anderson and Swett (*1*) as a means of effecting a separation of iron from copper in steel. The copper remains in the undissolved residue, which is then dissolved in nitric acid, and the resultant solution is electrolyzed. Unfortunately, many samples are difficult to dissolve by the mere use of sulfuric acid. Furthermore, Zinberg (*13*), who took great pains to exclude air during the solution process, found that some of the copper was invariably lost in the filtrate. He nevertheless claimed satisfactory results with samples containing from 0.5 to 5 per cent of copper by dissolving out the iron with sulfuric acid in a carbon dioxide atmosphere and igniting the residue to cupric oxide in a porcelain crucible.

Effective removal of the iron by the formation of ferric complexes has often been resorted to for analytical separations. Smith (*10*, *11*) and Fernberger and Smith (*4*) discussed the use of phosphoric acid for this purpose at an early date, although the proportions of iron to copper investigated were small as compared to actual conditions obtaining for present-day alloys. Falnberg and Troitzkaya (*3*) used potassium hydrogen fluoride for the formation of the FeF_6^{---} complex, although they did not investigate the feasibility of subsequent electrolytic removal of copper from such a solution. Hoar (*7*) prevented the interference of ferric ion with the colorimetric determination of copper by using citric acid, ammonia, and gum arabic, or sodium pyrophosphate.

Method

The procedures used involve relatively rapid dissolution of the sample steel, combined with the addition of phosphate or fluoride ion to tie up any ferric ion that may be formed. The resultant solution is then electrolyzed at a rather high current density while being maintained at a relatively low temperature.

APPARATUS. The electroanalyzer used for plating the copper was a rather old Fisher, single-spindle model which has been in use for many years in one of the advanced undergraduate laboratories.

For electrolysis of the copper-bearing solution the vessel shown in Figure 1 (obtainable from the Fisher Scientific Company, Pittsburgh, Penna.) has been found most satisfactory. Circulation of ice water through the outer jacket during passage of the current permits the use of high current densities without overheating the solution. With this cell, after complete deposition of the metal, the electrodes may be conveniently washed by opening the stopcock and gradually displacing the electrolysis solution with distilled water until zero current is registered on the ammeter.

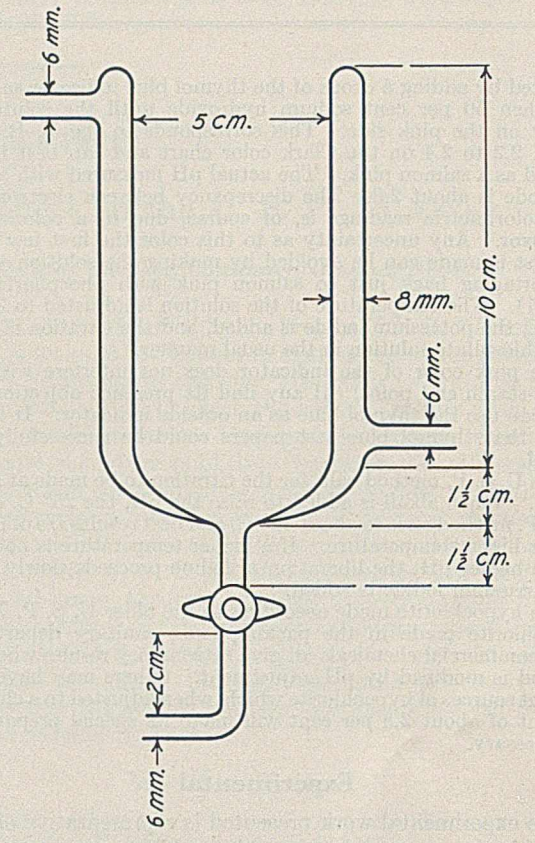

FIGURE 1. DIAGRAM OF APPARATUS

PROCEDURE. For relatively easily soluble samples the following procedure was used:

A sample between 1 and 5 grams (depending upon the copper concentration) was weighed into a 400-ml. beaker. Five milliliters each of concentrated sulfuric and sirupy phosphoric acid were added, followed by 250 ml. of distilled water. Gentle warming for 15 to 30 minutes usually effected solution. The resultant solution was then filtered into the electrolysis vessel, 5 ml. of a saturated disodium phosphate and 5 ml. of a 20 per cent ammonium sulfate solution were added, and the solution was electrolyzed at 3 volts and 2.5 to 3.5 amperes for one hour. The anode stirrer rotated at 500 r. p. m. For samples more difficult to dissolve, 5 ml. of nitric acid were initially added with the sulfuric acid. When reaction ceased the solution was evaporated until crystallization started, then cooled, diluted to 250 ml., and filtered.

An alternative procedure which also yielded satisfactory results, especially with samples ordinarily difficult to dissolve, is the following: The sample (from 1 to 5 grams) is weighed into a 100-ml. platinum dish. An acid mixture consisting of 5 ml. each of concentrated sulfuric, nitric, and hydrofluoric acids and 20 ml. of distilled water is slowly added. All samples that have been tried up to the present appear to dissolve very rapidly. After reaction has appeared to cease, the solution is gently warmed for 5 minutes and then diluted, filtered, and placed in the electrolysis vessel.

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TABLE I. DETERMINATION OF COPPER

Sample	B. of S. Value	Average Deviation Phosphate method	Average Deviation Fluoride method	Other Major Constituents Present
	%	%	%	%
115, Cu-Ni-Cr cast iron	6.44	6.46 ± 0.06 (14 detns.)	6.45 ± 0.02 (11 detns.)	Cr (2.17) Si (1.60) Ni (15.89)
5g, cast iron	1.44	1.44 ± 0.02 (7 detns.)	1.43 ± 0.01 (7 detns.)	Si (1.84)
13c, open-hearth steel	0.165	0.169 ± 0.003 (7 detns.)	0.160 ± 0.02 (6 detns.)	
73, stainless steel	0.033	0.033 ± 0.002 (4 detns.)	0.038 ± 0.00 (4 detns.)	Cr (13.93)
106, Cr-Mo-Al steel	0.142		0.140 ± 0.01 (3 detns.)	Cr (1.29) Al (1.06)
125, high-silicon steel	0.06		0.067 ± 0.007 (2 detns.)	Si (4.98)

Discussion

The temperature of the solution within the electrolysis vessel has been easily maintained at 10° C. or lower, even with the use of 6 amperes with a gauze electrode of approximately 30 sq. cm. surface. This prevents the deleterious effects of nitric acid and high temperatures suggested by Pan (*8*) and Geloso and Deschamps (*6*), who found that above 30° C. copper could not be deposited quantitatively from an acid solution. With cooling, a current efficiency of 66 per cent was obtained with a 3-ampere current, whereas under the same conditions but without cooling the efficiency (determined on synthetic solutions) was found to be 44 per cent. Using a solution with approximately equivalent amounts of acidified ferric and cupric sulfates, no copper could be obtained by plating for 40 minutes from the uncooled cell (the temperature reaching 38°), whereas at 8° in the same time 0.3824 gram of copper was obtained.

Results

Very gratifying results were obtained with the above methods on the National Bureau of Standards samples available. The results obtained in successive analyses are shown in Table I.

The averages and deviations are based on all the analyses made for each sample and are not averages of picked results.

Analyses made for M. C. Schwartz of the Gulf States Utilities, Baton Rouge, La., on private samples also checked well with results obtained in his laboratory by the copper sulfide-oxide method.

Conclusions

An electrolytic method for the quantitative determination of copper in ferroalloys has been found to yield satisfactory results on Bureau of Standards samples representing a wide range of copper concentrations. Using a special cell for temperature control of the electrolysis solution and either fluoride or phosphate ion for suppressing the effect of ferric ion on copper deposits, the copper may be plated without previous chemical separation. By this method the time-consuming filtration of hydroxide or sulfide precipitates is obviated.

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Identification of Primary Aliphatic Amides as Oxalates

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IT HAS long been known that amides will form salts with certain acids—for example, hydrochlorides are formed when dry hydrogen chloride is passed into dry ether solutions of amides. Urea oxalate is a water-stable compound of this type. Titherley (*2*) prepared a benzamide oxalate (2 amide to 1 acid) and a benzamide succinate (2 amide to 1 acid) in water.

The present investigation was undertaken to determine whether the common primary aliphatic amides would form stable compounds with oxalic acid. In the early stages of the work unsuccessful attempts were made to prepare the compounds in water. Although four different amides were tried (formamide, acetamide, propionamide, *n*-valeramide) an identical product resulted in each case—ammonium tetroxalate. The expected compound formation took place only under anhydrous conditions.

Table I lists the compounds prepared with their analytical data. The same products were obtained when the reagents were mixed in the ratio of 1 acid to 2 amide or 1 acid to 1 amide. The acetamide and propionamide derivatives were stable enough to have their molecular weights checked by the Rast (*1*) camphor method. Widely varying results were obtained with the other compounds.

Ethyl acetate was found to be a satisfactory crystallizing

TABLE I. COMPOUNDS PREPARED

Oxalate	Ratio of Amide to Acid	Nitrogen Calcd.	Nitrogen Found	Melting Point (Cor.)	Equivalent Weight[a] Calcd.	Equivalent Weight[a] Found[b]	Equivalent Weight[a] Found[c]
		%	%	° C.			
Formamide	2:1	15.58	15.61	107.4–107.7	90	91	90
Acetamide	1:1	9.39	9.37	127.3	75	75	76
Propionamide	1:1	8.58	8.54	80.8–81.0	82	81	81
n-Butyramide	2:1	10.59	10.35	65.9–66.2	132	133	132
n-Valeramide	2:1	9.58	9.41	61.1–61.4	146	145	146
n-Caproamide	2:1	8.75	8.11	71.1–71.3	160	161	158

[a] Molecular weights: acetamide calcd. 149, found 150; propionamide calcd. 163, found 160.
[b] NaOH titration.
[c] $KMnO_4$ titration.

solvent for all the compounds with the exception of the unusually soluble *n*-butyramide derivative.

Numerous unsuccessful attempts were made to prepare a derivative of isobutyramide.

Experimental

AMMONIUM TETROXALATE. Place in a test tube 1 gram of amide, 1 gram of oxalic acid (any ratio of amide to acid appears to produce the same result), and 5 ml. of water. Heat the mixture in a boiling water bath for 5 minutes. Crystallize the ammonium tetroxalate by placing the tube in an ice bath. Melting point 129.5–130.5° C. corrected.

AMIDE OXALATES. Place in an Erlenmeyer flask 1 gram of amide, the calculated quantity of anhydrous oxalic acid (based on the ratio of amide to acid in the compound being prepared), and 2 ml. of ethyl acetate. Warm the mixture to 60° to 65° C. in a water bath and add ethyl acetate 1 ml. at a time until no further solution is perceived. The time involved in the heating and the addition of the ethyl acetate need not exceed 10 minutes. Two to 20 ml. of ethyl acetate may be needed, depending on the amide used. At this point the solution may be cloudy or may contain a small amount of an insoluble residue of an undetermined nature. Filter the hot solution and cool the filtrate in an ice bath until the product crystallizes. The derivatives may be recrystallized, if necessary, from a minimum quantity of ethyl acetate at 60° to 65° C. Dry the crystals in a desiccator filled with sulfuric acid.

Increasing the time of heating or raising the temperature tends to increase the quantity of the undesirable insoluble residue mentioned above.

ANALYTICAL DATA. Equivalent weights were determined by titration with sodium hydroxide and with potassium permanganate according to the usual procedures. Nitrogen was determined by treating the compounds with sodium hydroxide, collecting the ammonia evolved in standard acid, and titrating the excess acid with standard base. The amides above propionamide showed increasing resistance toward hydrolysis with sodium hydroxide. The low value for the caproamide derivative was obtained after 6 hours' digestion with the usual Kjeldahl mixture of sulfuric acid, copper sulfate, and potassium sulfate.

These compounds should be particularly useful in qualitative organic analysis, since they are easily and quickly prepared, are stable in a dry form, and may be titrated with a base or with potassium permanganate.

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Studies of Organic Reagents and Methods Involving Their Use

Indirect Volumetric Determination of Metals Precipitated by Organic Reagents of the Oxime Type

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THE volumetric methods that have previously been developed for the indirect estimation of metals in the precipitates which they form with various oximes have been based upon the decomposition of the compound to yield hydroxylamine, and the indirect volumetric estimation of the latter by a permanganate procedure. For example, copper salicylaldoxime may be decomposed by heating with acid in the presence of an excess of a ferric salt. The salicylaldehyde volatilizes and the hydroxylamine is oxidized by ferric ion to yield nitrous oxide; the equivalent quantity of ferrous ion which is formed in the reaction is titrated with standard potassium permanganate. Tougarinoff (*13*) devised this method and reported good results with both copper salicylaldoxime and nickel dimethylglyoxime. The process has also been applied to copper α-benzoin oxime (*4*).

The method presented here is essentially a bromate-arsenite method for hydroxylamine that is applicable to the estimation of metals in various types of oxime precipitates. Data are presented upon the application of the process to copper salicylaldoxime, copper α-benzoin oxime, and nickel dimethylglyoxime. The volumetric process is believed to be of general utility in the estimation of oximes or in the indirect estimation of metals which are precipitated by the various oximes. Since ferric ion oxidizes hydroxylamine only to the nitrous oxide stage, whereas bromate oxidizes hydroxylamine to form nitrate, there is a more favorable ratio of standard solution used per unit weight of metal in the latter method. With certain types of oximes—e. g., salicylaldoxime—there is possible oxidation or bromination of the aldehyde that is formed in the decomposition of the precipitate, with consequent gain in sensitivity of the method.

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Materials and Apparatus

Potassium bromate of Merck's reagent grade was twice recrystallized from water, dried at 150° C., pulverized, and heated several hours longer at the same temperature. Solutions prepared from this stock were standardized against arsenious oxide from the National Bureau of Standards according to a standard procedure (*7*). Two bromate solutions thus prepared had normalities of (*a*) 0.1155, 0.1156, and 0.1155, average 0.1155; and (*b*) 0.1239, 0.1238, and 0.1239, average 0.1239.

Arsenious Acid. Arsenic trioxide of reagent grade was dissolved in sodium hydroxide solution, then acidified with hydrochloric acid. Solutions thus prepared were standardized by titration with previously standardized potassium bromate solution.

Hydroxylamine hydrochloride, salicylaldoxime (m. p. 53.5–55° C. uncorrected), and α-benzoin oxime (m. p. 149–151° C. uncorrected, *3*) were from the Eastman Kodak Company. Dimethylglyoxime obtained from both Merck and Kahlbaum was used in the form of a 1 per cent solution in alcohol. The salicylaldoxime solution contained 1 gram of the reagent per 100 ml. of 5 per cent alcohol.

Copper Sulfate. Merck's reagent grade copper sulfate sufficient to give about 2 grams of copper per liter was used for a copper solution, which was standardized electrolytically (*14*). Found per 25 ml.: 0.0498, 0.0498, 0.0497 gram.

Nickel Solution. Nickel nitrate of reagent grade was used to prepare solutions which were standardized by the gravimetric dimethylglyoxime procedure (*5*). Found for solution *a:* 0.0101, 0.0100, 0.0101, and 0.0101 gram per 5.00 ml.; for solution *b:* 0.0112, 0.0112, and 0.0112 gram per 10.00 ml.

Calibrated volumetric ware and weights were used. In

TABLE I. ESTIMATION OF HYDROXYLAMINE BY BROMATE-ARSENITE PROCEDURE

NH_2OH Present	0.1 N $KBrO_3$ Required	NH_2OH Found	Difference
Mg.	*Ml.*	*Mg.*	*Mg.*
1.80	3.25	1.79	−0.01
1.80	3.26	1.79	−0.01
1.80	3.32	1.83	0.03
9.28	16.85	9.27	−0.01
9.28	16.97	9.34	0.06
9.28	16.92	9.31	0.03
19.07	34.65	19.07	0.00
19.07	34.54	19.00	−0.07
19.07	34.51	18.99	−0.08

measuring some of the smaller volumes of solutions a calibrated 5.000-ml. microburet was used.

Bromate-Arsenite Procedure for Hydroxylamine

The estimation of hydroxylamine with bromate was tested under two sets of conditions at an acid concentration of 3 to 4 N. In the first a bromate-bromide mixture was used for the oxidation (*12*), while in the second bromate alone was allowed to act in the 3 to 4 N hydrochloric acid solution (*8*). In each case the process was completed by the potassium iodide–thiosulfate procedure for the estimation of the excess bromate.

For the purposes of this study the potassium iodide–thiosulfate method for the back-titration was found to be undesirable in the presence of copper, and hence the excess of bromate was reduced by adding a measured volume of standard arsenious acid; the excess of the latter was titrated with standard potassium bromate, using methyl orange indicator. The reactions are:

$$BrO_3^- + NH_2OH = NO_3^- + Br^- + H_2O + H^+$$

and

$$BrO_3^- + 3As^{III} + 6H^+ = Br^- + 3As^V + 3H_2O$$

The hydroxylamine is first oxidized by a measured excessive quantity of standard bromate in 3 to 4 N hydrochloric acid solution. The maximum amount of hydroxylamine that should be present in any determination is 20 mg.

A solution of hydroxylamine hydrochloride was prepared to test the validity of the bromate-arsenite procedure. This solution was standardized by titration with freshly standardized sodium hydroxide solution, using phenolphthalein indicator (*9*). The sodium hydroxide was standardized against constant boiling hydrochloric acid. The aliquots were 25.10 ml. each, and the results were:

0.0836 N NaOH used, ml.	17.09, 17.06, 17.11, 17.10
NH_2OH, mg. per 25.1 ml.	47.19, 47.11, 47.25, 47.22
	Av. 47.19

BROMATE-ARSENITE PROCEDURE. Aliquot portions of the hydroxylamine hydrochloride solution were placed in 250-ml. glass-stoppered Pyrex Erlenmeyer flasks, and 50 ml. of cold 6 N hydrochloric acid added to each. A measured excessive volume of standard potassium bromate was then added and the flask was quickly stoppered. In general, about 10 ml. of the bromate were added in excess. After 15 minutes the flask was cooled and a measured volume of standard arsenious acid was quickly poured in from a beaker into which it had been pipetted. It was later found advisable when dealing with the salicylaldoxime precipitates to add all reagents through a separatory funnel. After all the arsenite had been washed into the flask, the excess arsenite was titrated with the standard bromate, using methyl orange indicator (0.1 per cent aqueous solution). Each milliliter of 0.1 N potassium bromate is equivalent to 0.00005505 gram of hydroxylamine. Some results obtained by this procedure are given in Table I.

While longer than the method previously mentioned (*8*), the bromate-arsenite procedure is more sensitive, employs a more stable reducing solution, and is not interfered with by certain metallic ions which vitiate the iodide-thiosulfate method.

General Procedure

APPLICABLE TO METALLO-ORGANIC PRECIPITATES FORMED BY OXIMES. The compound is precipitated under the well-established conditions, after prior removal of interfering substances (*3, 4, 6*), and the precipitate is collected and washed on a suitable filtering crucible (No. 4 Jena glass, Gooch crucible, etc.). The precipitate is dissolved in 25 ml. of concentrated hydrochloric acid and drawn by suction into the flask or bottle in which the titration is to be made. The crucible is washed with 25 ml. of water. If necessary, more acid and more water are used in 1 to 1 ratio. The vessel is then fitted with a stopper carrying a separatory funnel and a tube provided with a 5-cm. (2-inch) piece of rubber tubing and a pinchclamp. An amount of bromate estimated to be about 10 ml. in excess is then added through the separatory funnel. If necessary, water may be added to bring the acid concentration down to 3 to 4 N.

After mixing and standing 15 minutes, a measured excess of arsenite treated with 1 to 2 drops of methyl orange is placed in the separatory funnel and the flask or bottle is cooled under a tap, so that a good part of the arsenite is drawn in when the stopcock of the funnel is opened. When the red color of the methyl orange persists, after shaking the vessel, the remainder of the arsenite and the washings are allowed to flow in by opening the pinchclamp. The stopper and funnel are then removed, and the excess of arsenite is titrated with bromate, adding more methyl orange if necessary. The most critical part of the procedure is the prevention of the escape of bromine by adding the arsenite to the closed system after the reaction.

Over the permissible range of hydroxylamine (0 to 20 mg.) the method is adaptable to determining semimicroquantities of metals with ordinary volumetric technique. If milligram quantities of metal are to be estimated, microburets or weight burets should be used for the measurements.

TABLE II. INDIRECT DETERMINATION OF COPPER IN ITS SALICYLALDOXIME COMPOUND

Copper Present	0.1 N $KBrO_3$ Used	Copper Found	Error
Mg.	*Ml.*	*Mg.*	*Mg.*
0.93	2.190	0.99	0.06
1.75	3.905	1.77	0.02
1.75	3.957	1.80	0.05
1.99	4.36	1.98	−0.01
1.99	4.43	2.01	0.02
6.81	14.86	6.75	−0.06
6.81	14.91	6.77	−0.04
6.81	15.02	6.82	0.01
8.03	17.33	7.87	−0.16
8.03	17.77	8.07	0.04
8.03	17.67	8.02	−0.01
17.70	39.07	17.74	0.04
17.70	39.16	17.80	0.10

Indirect Determination of Copper in Salicylaldoxime Precipitate

The copper is precipitated in the usual way (*6*) from a solution containing acetic acid and sodium acetate (pH about 3 and not lower than 2.6). The process is a separation of copper from silver, mercuric, cadmium, arsenious, ferric, cobalt, nickel, and zinc ions (*6*). After washing with water, the precipitate is handled by the general procedure. In this instance a definite number of equivalents of oxidant is consumed per gram-atom of copper, but no simple equation can be written for the process, which must be regarded as an empirical one.

Free bromine is present in the solution during the course of the process:

$$NH_2OH + BrO_3^- = NO_3^- + Br^- + H_2O + H^+$$

and

$$BrO_3^- + 5Br^- + 6H^+ = 3Br_2 + 3H_2O$$

and some bromination of the organic part of the compound would be expected to occur. The partially oxidized cream-white product which precipitates immediately after the addi-

tion of the bromate contains bromine and nitrogen, the latter detected by Feigl's test (*2*). Under the conditions specified in the general procedure, 14 equivalents of oxidant are used per mole of copper salicylaldoxime, which is more than that required for the hydroxylamine (2 moles) that might be liberated from the compound, but less than would be needed for complete bromination of the salicylaldehyde as well. Some typical results are given in Table II.

With 20 mg. of copper present the process gives erratic results which tend to be about 0.7 mg. high. The milliequivalent weight of copper is $\frac{63.57}{14{,}000} = 0.004541$ gram and hence 1 ml. of 0.1 *N* potassium bromate equals 0.4541 mg. of copper.

The copper in a bronze from the National Bureau of Standards was determined after the removal of tin by a standard procedure (*15*). The average values on aliquots from two samples were 88.22 and 88.36 as compared with the certificate value of 88.33 per cent copper.

In experiments not detailed here it was found that nickel salicylaldoxime [$Ni(C_7H_6O_2N)_2$] like the analogous copper compound requires 14 equivalents of the bromate per mole, whereas the lead compound which has the formula $PbC_7H_5O_2N$ requires 8 equivalents of potassium bromate per mole.

The following observations regarding the oxidation of the copper salicylaldoxime have been made: After oxidation the filtrate from the white precipitate contains nitrate and not nitrite (*1*). The white precipitate has a molecular weight of about 240 (*11*). Roughly one fourth of the nitrogen of the original compound appears as nitrate in the filtrate from the white precipitate. If bromide is added along with the bromate, only 12 equivalents of bromate are consumed in the process of oxidation. If the hydrochloric acid solution of the copper salicylaldoxime is boiled before adding the bromate, a larger consumption of oxidizing agent occurs. It is therefore evident that an established procedure must be strictly adhered to, in view of the possibility of varying the stoichiometry.

Table III. Indirect Determination of Nickel by Bromate-Arsenite Procedure

Ni Present	0.1 *N* $KBrO_3$ Used	Ni Found	Error
Mg.	*Ml.*	*Mg.*	*Mg.*
1.10	4.69	1.15	0.05
1.10	4.64	1.13	0.03
1.10	4.71	1.15	0.05
5.50	22.92	5.60	0.10
5.50	22.85	5.59	0.09
5.50	22.15	5.42	−0.08
11.00	44.74	10.94	−0.06
11.00	44.80	10.95	−0.05
11.00	44.69	10.93	−0.07

Indirect Determination of Nickel after Precipitation with Dimethylglyoxime

Previous volumetric methods for this purpose have involved hydrolysis and oxidation of the hydroxylamine by ferric salt, followed by titration of the ferrous salt that is formed with potassium permanganate (*13*), or alternatively titration of the nickel by the cyanometric method (*10*). The bromate-arsenite method is applied after nickel has been precipitated with dimethylglyoxime in the usual manner and washed (*5*). In this case the hydrochloric acid solution of the precipitate is boiled 5 minutes for milligram quantities of nickel present or 10 minutes with 0.01 gram of nickel present, then cooled to room temperature before adding the standard bromate solution. The equivalent weight of nickel is one twenty-fourth of the atomic weight. Hence 1 ml. of 0.1 *N* potassium bromate equals 0.2445 mg. nickel. Some typical results are given in Table III.

Table IV. Indirect Volumetric Determination of Copper after Precipitation with α-Benzoin Oxime

Copper Present	0.1 *N* $KBrO_3$ Used	Copper Found	Error
Mg.	*Ml.*	*Mg.*	*Mg.*
3.40	3.30	3.50	0.10
3.40	3.33	3.53	0.13
6.80	6.50	6.88	0.08
6.80	6.42	6.80	0.00
17.20	16.50	17.48	0.28
17.20	16.34	17.31	0.11
17.20	16.30	17.27	0.07

Indirect Determination of Copper after Precipitation with α-Benzoin Oxime

The copper is precipitated from an ammoniacal solution, this procedure being specific for copper in the presence of cobalt, nickel, ferric iron, aluminum, and lead if sufficient tartrate is present (*3*). After washing with dilute ammonia (1 ml. of concentrated plus 100 ml. of water) and with hot alcohol the precipitate is dissolved with hot concentrated hydrochloric acid, the remainder of the technique being as described under general procedure, with the exception that the solution is boiled 5 to 10 minutes and cooled before adding the standard potassium bromate.

Six equivalents of oxidant are consumed per gram-atom of copper and the equivalent weight is $\frac{63.57}{6} = 10.595$. Hence 1 ml. of 0.1 *N* potassium bromate is equivalent to 1.0595 mg. of copper. Typical results are given in Table IV.

This method is inherently less sensitive than the salicylaldoxime method, as less than half as much bromate is consumed in the oxidation of the decomposition products of the copper α-benzoin oxime. It is very difficult to wash the excess of reagent out of the precipitate, which may account for the tendency of the results to be slightly high.

Summary

A bromate-arsenite procedure has been tested for the estimation of hydroxylamine and found accurate.

The hydroxylamine that is liberated from nickel dimethylglyoxime or from copper α-benzoin oxime may be determined by the bromate-arsenite method to give an indirect estimation of copper or nickel.

Copper salicylaldoxime is oxidized in a reproducible manner under strictly controlled conditions by the bromate-arsenite procedure. One gram-atom of copper requires 14 equivalents of standard bromate in the titration. The method is suitable for the estimation of semimicroquantities of copper.

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Determination of Gossypol in Crude Cottonseed Oil

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SMITH and Halverson (*5*) recently pointed out a possible source of error in the pyridine-aniline method for the determination of gossypol, due to the presence of pyridine of crystallization in the precipitate of dianilinogossypol. With reference to the original paper (*2*) on the method, this criticism probably is justified, since the proposed procedure of drying the precipitate (1 hour's heating at 100° C.) did not assure quantitative removal of the pyridine. However, a modification (*3*) published the following year, provided for heating the dianilinogossypol to constant weight at 100° C., and the present paper demonstrates that the pyridine of crystallization retained by the precipitate can be removed quantitatively, without appreciable decomposition of the dianilinogossypol. While this removal may be accomplished at 100° C., it can be effected more rapidly and conveniently at a higher temperature. Also, the dianilinogossypol precipitated with 2 molecules of pyridine of crystallization can be dried to constant weight at a low temperature, without loss of pyridine, so that the dianilinogossypol-dipyridine may be weighed as such and calculated to gossypol by means of the conversion factor 0.627.

In the course of this work another minor modification of the original method was found to give increased accuracy and reliability. The addition of a small amount of pyridine to the petroleum ether used for washing the dianilinogossypol-dipyridine reduced the solubility of the precipitate to an appreciable extent, making it possible to recover up to 98 per cent of the gossypol from a 0.2 per cent solution in oil. These improvements are embodied in a revision of the method presented herein, and the experimental data forming the bases for these changes are summarized.

In a recent paper, Campbell, Morris, and Adams (*1*) described the preparation of a dipyridine salt of gossypol, $C_{30}H_{30}O_8.2C_5H_5N$. On account of the possible interference of such a compound in the determination of gossypol by the pyridine-aniline method, a few notes on the precipitation of gossypol from pyridine solutions, in the absence of aniline, are included in the present paper. In general, pyridine alone would not precipitate gossypol from crude cottonseed oil, and the solubility of the pyridine salt was sufficient to preclude contamination of the dianilinogossypol-pyridine complex. The solubility of gossypol in pyridine was found to be 13.3 per cent by weight, and upon dilution of this saturated solution with petroleum ether, a crystalline precipitate of $2C_{30}H_{30}O_8.5C_5H_5N$, rather than the dipyridine salt, was obtained.

Experimental

EFFECT OF HEATING CONDITIONS ON COMPOSITION OF DIANILINOGOSSYPOL PRECIPITATED FROM COTTONSEED OIL WITH A MIXTURE OF PYRIDINE AND ANILINE.—A stock solution of purified gossypol in refined cottonseed oil was prepared by adding an ethereal solution of gossypol to the oil, followed by complete removal of the solvent under reduced pressure. The gossypol concentration was adjusted to 0.4 per cent, so that the 25-gram samples taken for analysis contained exactly 100.0 mg. of gossypol. The gossypol had been purified by recrystallization from a petroleum ether–ether mixture, and was a bright yellow, microcrystalline powder having a molecular weight of 518 (titration with 0.25 *N* sodium hydroxide), and melting point of 184° C. A further indication of the purity of this preparation is the crystal form, shown in Figure 1. The pyridine and aniline employed in this work were C. P. grade (Baker), and the petroleum ether was Skellysolve F (boiling range 35° to 60°).

With the exception of heating conditions and details outlined in Table I, the general analytical procedure was that described in a previous paper (*3*). Unless otherwise stated, all the gossypol analyses given in the tables are averages of three determinations.

Samples 1 to 3, Table I, show that the dianilinogossypol-pyridine complex reaches constant weight in 2 hours at 60° C., and that the nitrogen content remains fairly constant. The pyridine content of the precipitate was calculated from the nitrogen analysis, by means of the following equation:

$$17.72\,P + 4.19(100 - P) = 100\,N$$

or

$$P = 7.41\,N - 30.9$$

where P = per cent pyridine, N = per cent nitrogen, and 17.72 and 4.19 represent the nitrogen percentages in pyridine and dianilinogossypol, respectively.

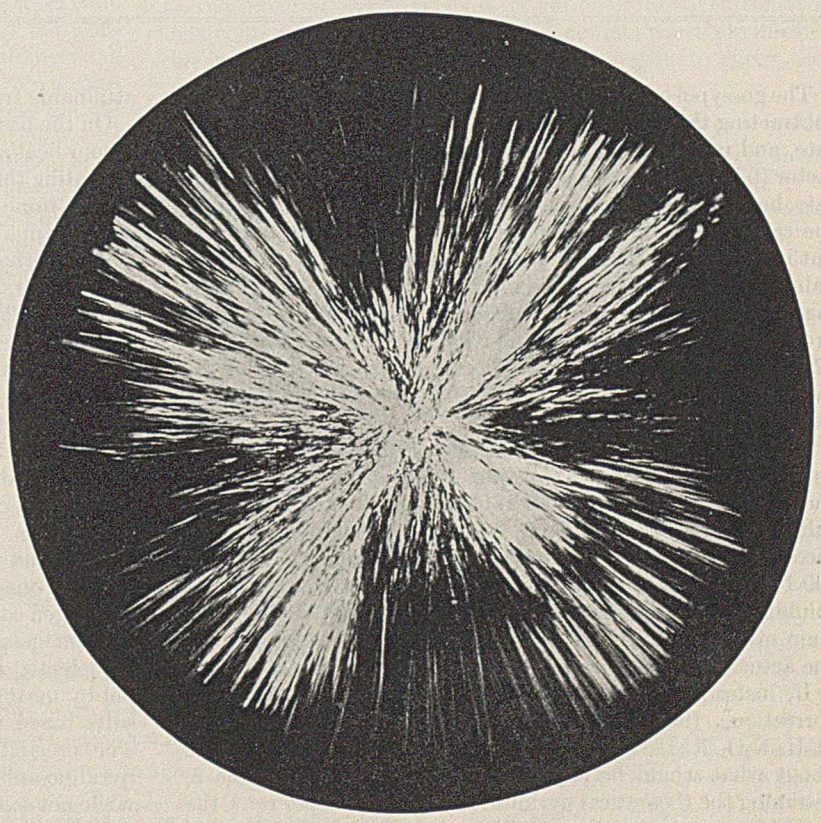

FIGURE 1. PURIFIED GOSSYPOL
× 450, m. p. 184° C., crystallized from a mixture of ether and petroleum ether

TABLE I. EFFECT OF HEATING CONDITIONS ON PYRIDINE CONTENT OF PRECIPITATE

(Samples 1 to 11, inclusive, contain 100.0 mg. of pure gossypol (molecular weight 518) in 24.90 grams of refined cottonseed oil; 7.5 ml. of pyridine-aniline reagent (4 to 1) and 50 ml. of petroleum ether added, allowed to precipitate 7 days at room temperature. Samples 12 to 15, inclusive, represent 100-gram samples of typical hot-pressed crude cottonseed oil, no added gossypol.)

Sample	Heating Conditions: Temperature	Heating Conditions: Time	Weight of Precipitate	Nitrogen Content	Pyridine Content Calcd. from Nitrogen	Gossypol Found: Corrected for analyzed pyridine content	Gossypol Found: Precipitate weighed as $C_{42}H_{40}N_2O_6.2C_5H_5N$ (factor 0.627)
	° C.	Hours	Mg.	%	%	Mg.	Mg.
1	60	1	157.0	6.60	18.0	99.9	98.4
2		2	155.0	6.56	17.7	99.1	97.1
3		3	155.0	6.54	17.6	99.4	97.1
4	100	3	134.9	4.79	4.4	99.9	...
5		5	128.3	4.48	2.2	97.3	...
6		7	126.8	4.30	0.8	97.5	...
7		9	124.8	4.11	None	96.7	...
8		12	124.5	4.12	None	96.5	...
9	160	0.5	125.3	4.11	None	96.9	...
10		1	124.6	4.08	None	96.6	...
11		2	125.0	4.06	None	96.6	...
12 Crude cottonseed oil 117	60	2	197.6	6.73	19.0	124.1	123.8
13 Crude cottonseed oil 117	160	1	158.7	4.29	None	123.0	...
14 Crude cottonseed oil 300	60	2	174.4	6.62	18.2	110.4	109.3
15 Crude cottonseed oil 300	160	1	146.4	4.07	None	113.5	...

TABLE II. EFFECT OF WASHING PROCEDURE IN PYRIDINE-ANILINE METHOD

(Each sample contained 100.0 mg. of gossypol in 49.90 grams of cottonseed oil; 70 ml. of Skellysolve F, 6 ml. of pyridine, and 1.5 ml. of aniline were added, mixed, and allowed to stand 5 days.)

Experiment No.	Volume of Wash	Composition of Wash Solution: Skellysolve F: Wt. of precipitate[a]	Skellysolve F: Nitrogen[b] content	Skellysolve F: Gossypol[c] found	Skellysolve F Containing 2% Pyridine: Wt. of precipitate	Skellysolve F Containing 2% Pyridine: Nitrogen content	Skellysolve F Containing 2% Pyridine: Gossypol found
	Ml.	Mg.	%	Mg.	Mg.	%	Mg.
1	60	120.6	..	93.5	127.0	4.09	98.4
2	120	119.8	4.08	92.8	126.2	4.07	97.7
3	180	117.2	4.04	90.8	124.0	4.22	96.1
4	240	108.8	..	84.3	123.6	4.12	95.8
Calculated values for 100% recovery		129.0	4.19	100.0	129.0	4.19	100.0

[a] All precipitates heated one hour at 160° C., and weighed as dianilinogossypol.
[b] Nitrogen values are given to show complete removal of pyridine from dianilinogossypol-dipyridine by heating 1 hour at 160° C. [c] Weight of precipitate multiplied by factor 0.775.

The gossypol content of the precipitate is then calculated by subtracting the weight of pyridine from the weight of precipitate, and multiplying the difference by the dianilinogossypol factor (0.775). The analyzed pyridine content of the precipitate, heated to constant weight at 60° C., is slightly lower than the calculated value (19.2 per cent) for $C_{42}H_{40}N_2O_6.2C_5H_5N$, but it is probable that the discrepancy is due to low nitrogen values returned by the Kjeldahl method on samples containing pyridine compounds. Three samples of purified, anhydrous pyridine, analyzed by the Kjeldahl method, gave 17.39, 17.29, and 17.69 per cent of nitrogen, respectively, for an average of 17.46 per cent, which is 0.27 per cent lower than the calculated value of 17.72 per cent.

As further evidence that the dianilinogossypol-pyridine complex, upon heating to constant weight at 60° C., contains the theoretical percentage (19.2) of pyridine for the dipyridine salt, it will be noted in Table I that the calculated gossypol recovery, based on the analyzed pyridine content, is too high (99.1 to 99.9 per cent recovery). Owing to the appreciable solubility of the dianilinogossypol in the mixture of oil, petroleum ether, pyridine, and aniline from which it precipitates, the actual recovery cannot greatly exceed 98 per cent.

If, instead of using the analyzed pyridine percentage for correction, the theoretical value (19.2 per cent) for $C_{42}H_{40}N_2O_6.2C_5H_5N$ be used, the amount of gossypol found is about what would be expected—for example, on sample 2, assuming the theoretical pyridine content of 19.2 per cent, the factor for converting the dianilinogossypol-dipyridine salt to gossypol is 0.627, and the gossypol recovery is 97.1 mg.

Samples 4 to 8, Table I, show that when the dianilinogossypol-dipyridine is held at 100° C., the pyridine is driven off slowly but quantitatively, constant weight being attained in about 9 hours, and the nitrogen content (4.12 per cent) indicates that all the pyridine has been expelled, without decomposition of the residual dianilinogossypol. In all cases (samples 7 to 11, inclusive) where the nitrogen content of the precipitate is equal to or less than that of pure dianilinogossypol (4.19 per cent), the absence of pyridine is assumed, and the weight of precipitate is converted to gossypol by means of the factor 0.775.

Employing a drying temperature of 160° C., constant weight is obtained in 30 to 60 minutes, and the nitrogen content of the residue (sample 10, 4.08 per cent) indicates that it is free from pyridine. Further evidence that the residue is dianilinogossypol is shown by the percentage recovery (96.6), since this figure was obtained by using the dianilinogossypol conversion factor (0.775), and is in agreement with the normal percentage recovery usually attainable from a dilute solution of gossypol in oil.

On the basis of these experiments, it is recommended that a 1-hour heat at160° C. be substituted for the former procedure of heating the precipitate to constant weight at 100° C. This change minimizes the danger of pyridine retention and shortens the time required for analysis.

An alternative procedure for handling the precipitate is to heat 2 hours at 60° C., leaving a residue of dianilinogossypol containing 2 molecules of pyridine, in which case the conversion factor is 0.627. However, owing to the possibility of incomplete removal of moisture and solvent at 60° C., it is usually preferable to drive off the pyridine of crystallization at 160° C. and weigh as dianilinogossypol.

Samples 12 to 15, inclusive, Table I, indicate that the precipitate of dianilinogossypol-dipyridine obtained from crude hot-pressed cottonseed oil may be treated as above, and either weighed as the dipyridine salt (factor 0.627), or heated to 160° C. and weighed as dianilinogossypol (factor 0.775).

While one of the samples heated to 160° C. (No. 13) shows a nitrogen content slightly higher than the calculated value for dianilinogossypol (4.19 per cent), no residual pyridine could be detected in the precipitate and, when calculated to gossypol by means of the factor 0.775, fair agreement with the results based on weighing the dipyridine salt were obtained. The precipitates formed in crude cottonseed oils with the pyridine-aniline reagent are invariably macrocrystalline, and while not so coarse and brightly colored as those formed in the stock solution of purified gossypol in refined oil, they are easy to wash and filter.

TABLE III. RATE OF PRECIPITATION OF GOSSYPOL FROM COTTONSEED OIL

(Samples contain 100.0 mg. of gossypol dissolved in 49.9 grams of refined cottonseed oil, 70 ml. of petroleum ether)

Sample	Precipitating Agent	Crystallization Time	Weight of Precipitate[a]	Gossypol Recovered (100.0 Mg. Present)
		Days	*Mg.*	*Mg.*
20	6 ml. of pyridine	14	No ppt.	None
21	7.5 ml. of pyridine-aniline (4 to 1)	1	107.0	82.9
22		2	117.6	91.1
23		3	121.0	93.7
24		4	122.4	94.8
25		5	123.4	95.6
26		7	125.0	96.9
27		14	125.2	97.0
28	1.5 ml. of aniline	1	46.6	36.1
29		2	48.0	37.2
30		3	60.2	46.6
31		4	67.0	51.9
32		5	80.0	62.0
33		7	96.0	74.4
34		14	103.0	79.8

[a] Heated 1 hour at 160° C., converted to gossypol by factor 0.775.

Modified Washing Procedure

The advantages of adding a small amount of pyridine to the petroleum ether used in washing the precipitated dianilinogossypol-pyridine complex are shown by Table II. An excessive amount of washing lowers the recovery, particularly when no pyridine is added to the wash. Depending upon the washing technique, the minimum volume of petroleum ether necessary to ensure complete transference of the precipitate from flask to filter, and complete removal of oil, will vary somewhat, but usually at least 50 ml. are required, and a wash volume of 60 ml. is recommended as a standard procedure. Nitrogen determinations were made on the washed precipitates to check on the possibility of error due to pyridine retention by the samples washed with the pyridine solution. However, after heating the samples 1 hour at 160° C., those washed with the pyridine solution have about the same nitrogen content as those washed with petroleum ether, so that no error is introduced by weighing as dianilinogossypol and converting to gossypol by the factor 0.775. The pyridine wash makes possible a recovery of 98.4 mg. of gossypol from a solution containing 100 mg. in 50 grams of oil, whereas the old method recovers only 93.5 mg. from the same solution.

The effect of pyridine on the rate and completeness of precipitation of dianilinogossypol from oil solution is well illustrated by the tests in Table III (samples 20 to 34). All the analyses in these tests were run under identical conditions, except for the omission of pyridine in the second series, so that the results are directly comparable. In the absence of pyridine, precipitation is relatively slow and incomplete, amounting to only 80 per cent recovery in 14 days (sample 34). With pyridine present, more than 80 per cent of the gossypol is precipitated the first day, and the reaction is virtually complete in 7 days, giving a recovery of 96.9 per cent. Extending the precipitation period to 14 days only increases the percentage recovery to 97.0, so that for all practical purposes a 7-day precipitation period is sufficient, in the presence of pyridine.

Pyridine Salts of Gossypol

Pyridine alone does not precipitate gossypol from dilute oil solutions, as shown by sample 20, Table III. Since the formation of an insoluble pyridine salt of gossypol has been reported in the literature (*1*), the solubility of gossypol in pyridine was studied further.

In the first experiment, the work referred to above was repeated. One hundred milligrams of pure gossypol were dissolved in 10 ml. of C. P. pyridine, and the solution was diluted with several volumes of petroleum ether. No precipitate formed on standing 14 days. The solubility of gossypol in pyridine was then determined, and found to be 13.3 per cent by weight, at 25° C. Upon dilution of this saturated solution with petroleum ether, a finely crystalline, greenish-yellow precipitate formed, which, upon heating 42 hours at 60° C., analyzed 5.18 per cent nitrogen.

The precipitate was still losing weight, though very slowly, at the end of the heating period, 2.095 grams losing 2.4 mg. in the last 3 hours. The nitrogen content of 5.18 per cent is equal to 29.2 per cent pyridine, or a ratio of 2.5 moles of pyridine per mole of gossypol, corresponding to the formula $2C_{30}H_{30}O_8.5C_5H_5N$. This substance was then heated at 100° C. until it approached constant weight (3 hours), whereupon the nitrogen content was reduced to 0.95 per cent, equivalent to 5.36 per cent of pyridine, slightly less than 0.5 mole per mole of gossypol (calculated for $C_{30}H_{30}O_8.{}^1/_2C_5H_5N$, 1.03 per cent of nitrogen). In view of the fact that the precipitate was still losing weight slowly when the nitrogen analysis was made, proof of the existence of the compound $C_{30}H_{30}O_8.{}^1/_2C_5H_5N$ is not claimed. Upon heating at higher temperatures, the gossypol darkened and decomposed while liberating the remainder of the pyridine.

From the above observations it is apparent that the comparatively high solubility of gossypol in pyridine precludes the interference of pyridine-gossypol salts in the precipitation of the dianilinogossypol-pyridine complex from cottonseed oil with a mixture of pyridine and aniline.

Another factor which affects the precipitation of gossypol from a dilute oil solution is solvent volume. Table IV shows that while this volume is not very critical, complete precipitation cannot be obtained in the absence of petroleum ether, nor can maximum recovery be obtained when the solvent volume is more than twice the sample volume. The solvent also serves the purpose of speeding up the filtration.

Inasmuch as many types of crude hot-pressed cottonseed oils contain appreciable amounts of nonfats which are insoluble in petroleum ether, it was thought advisable to check the

TABLE IV. EFFECT OF SOLVENT VOLUME ON PRECIPITATION OF GOSSYPOL FROM CRUDE COTTONSEED OIL

(50 grams of oil, 6 ml. of pyridine, 1.5 ml. of aniline, diluted with specified amounts of petroleum ether, allowed to precipitate 7 days)

Sample	Petroleum Ether (Skellysolve F)	Gossypol Found
	Ml.	%
60	None	0.055
62	35	0.098
64	70	0.096
66	105	0.093
68	140	0.074

TABLE V. EFFECT OF WASHING PROCEDURES ON PYRIDINE SALT OF DIANILINOGOSSYPOL PRECIPITATED FROM CRUDE COTTONSEED OIL

Sample		Washing Procedure[a]	Weight of Precipitate Dried to Constant Weight at 160° C.	Gossypol Found
			Gram	%
90	Crude cottonseed oil No. 3 (50.0 grams)	5 ml. of 95% alcohol, followed by 10 ml. of 50% alcohol and 10 ml. of water	0.0809	0.125
91	Crude cottonseed oil No. 3 (50.0 grams)	Three 10-ml. portions of hot 50% alcohol	0.0815	0.126
92	Crude cottonseed oil No. 3 (50.0 grams)	Three 10-ml. portions of hot water followed by 5 ml. of 95% alcohol	0.0823	0.128
93	Crude cottonseed oil No. 3 (50.0 grams)	Two 10-ml. portions of hot 50% pyridine	0.0836	0.130
94	Crude cottonseed oil No. 3 (50.0 grams)	10 ml. of hot 50% pyridine, 10 ml. of water, 10 ml. of 95% alcohol	0.0819	0.127
95	Crude cottonseed oil No. 3 (50.0 grams)	Two 10-ml. portions equal parts pyridine and alcohol	0.0854	0.132
96	Oil No. 3, plus 100 mg. of gossypol	Same as in 94	0.2220	0.344
97	Oil No. 3, plus 100 mg. of gossypol	Same as in 93	0.2210	0.342

[a] Initial wash with 60 ml. of Skellysolve F containing 2 per cent pyridine preceded above washes in every case.

presence of such compounds in the dianilinogossypol-dipyridine complex by washing with water, aqueous solutions of alcohol, and pyridine. Moreover, Smith in his revised method for the determination of gossypol in cottonseed meal (*4*) specifies an alcohol and water wash, and owing to the chance of water-soluble meal extractives being present in crude oil, a similar washing treatment was tested in the present method.

However, reference to Table V indicates that neither water, alcohol, nor pyridine washes change the weight of the precipitate to any significant degree.

The effect of adding purified gossypol to a crude oil prior to analysis is shown by samples 96 and 97, to which 0.2 per cent of gossypol was added. Taking 0.128 per cent as the average amount of gossypol found in the crude oil, samples 96 and 97 would be expected to return 0.328 per cent, whereas the actual recovery was 0.343 per cent. This increase in recovery may be explained by the seeding action of the added gossypol, whereby precipitation of dianilinogossypol is brought about soon after addition of the reagents, and a large number of crystal nuclei are formed to act as centers of subsequent crystal growth. In the absence of this added gossypol, precipitation does not start for several days after the addition of the pyridine-aniline, and even after the first appearance of the precipitate, crystal nuclei are not numerous enough to bring the precipitation to completion in 7 days. Precipitation is hastened by agitating the samples for a brief period each day after the initial appearance of crystals.

On the basis of the results of the foregoing experiments, the following revision of the pyridine-aniline method for the determination of gossypol in crude cottonseed oil is outlined.

TABLE VI. REPRODUCIBILITY OF DETERMINATIONS AT VARIOUS GOSSYPOL CONCENTRATIONS

	Sample	Gossypol Found %	Mean %	Percentage Deviation from Mean
1	Hot-pressed oil A	0.0036 0.0042	0.0039	7.7
2	Hot-pressed oil B	0.110 0.113	0.1115	1.4
3	Expeller oil E	1.370 1.402	1.386	1.1
4	Stock solution of 0.1% gossypol in cottonseed oil	0.095 0.096	0.0955	0.5

Proposed Modification of Pyridine-Aniline Method

Weigh 50 grams of filtered crude cottonseed oil in a 200-ml. wide-mouthed extraction flask and dilute to 110 to 130 ml. with petroleum ether (Skellysolve F). Add 10 ml. of a mixture of 4 parts pyridine and 1 part aniline, mix well by swirling, stopper loosely, and store in a warm place (35° to 40° C.) for several hours. Replace any solvent lost by evaporation, and hold the samples at room temperature for 7 to 14 days. To prevent undue loss of solvent during the precipitation period, tight-fitting stoppers provided with a capillary vent may be employed. In any case maintain the solvent volume close to its original value throughout the precipitation and agitate the samples once each day after precipitation has begun. Oils containing less than 0.1 per cent of gossypol should be allowed 14 days to precipitate.

Filter under suction on tared crucibles provided with medium asbestos mat, washing the crystals of dianilinogossypol-dipyridine onto the filter with petroleum ether containing 1 to 3 per cent of pyridine. Wash the crystals free from oil with this solvent mixture, keeping the total volume of wash below 100 ml. Drive off the pyridine of crystallization by heating the precipitate 1 hour at 160° C., and weigh as dianilinogossypol. If it is desired to weigh the precipitate as dianilinogossypol-dipyridine, heat 2 hours at 60° C. The factors for converting weights of dianilinogossypol and dianilinogossypol-dipyridine to gossypol are 0.775 and 0.627, respectively.

The above method will recover 95 to 98 per cent of the gossypol from a 0.1 per cent oil solution, which is about the average gossypol concentration encountered in analysis of hot-pressed cottonseed oils. The reproducibility of results is fairly good for this type of analysis, as shown by the results obtained on two commercial samples of hot-pressed oil, an expeller oil, and a stock solution of 0.1 per cent gossypol in refined cottonseed oil (Table VI).

Referring to sample 1, it will be noted that the results are not closely reproducible on oils containing less than 0.01 per cent of gossypol, because precipitation is relatively slow and incomplete. At 0.1 per cent and higher concentrations the method is satisfactory. Sample 4 indicates that better checks can be obtained on stock solutions of purified gossypol in refined oil than on corresponding concentrations of gossypol in crude oil (sample 2), probably because of the presence of gossypol degradation products and other nonfats in the crude oil. As recommended in an earlier paper (*2*) accuracy and reproducibility of results on oil samples containing less than 0.01 per cent of gossypol are improved by the addition of a known amount of pure gossypol to the oil being analyzed.

Summary

A mixture of pyridine and aniline is more effective than aniline alone in precipitating gossypol from crude cottonseed oil. A modification of the pyridine-aniline method, which recovers up to 98 per cent of gossypol from a 0.2 per cent solution in oil, is described.

The pyridine-aniline reagent precipitates dianilinogossypol containing 2 molecules of pyridine of crystallization. The pyridine is removed quantitatively, without decomposition of the residual dianilinogossypol, by heating the precipitate 1 hour at 160° C. The dianilinogossypol-dipyridine complex is fairly stable at 60° C., and after heating for 2 hours at this temperature, its composition corresponds closely to the formula $C_{42}H_{40}N_2O_6.2C_5H_5N$.

The addition of a small amount of pyridine to the petroleum ether used for washing the dianilinogossypol precipitate decreases the solubility of the precipitate, and gives higher recovery of gossypol from dilute oil solutions.

Pyridine alone will not precipitate gossypol from crude cottonseed oil. The solubility of gossypol in pyridine was found to be 13.3 per cent by weight at 25° C. A pyridine-gossypol complex having the formula $2C_{30}H_{30}O_8.5C_5H_5N$ is described.

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Jacketed Receiver for Vacuum Distillation

Our attention has been called by Baird and Tatlock (London), Ltd., to the similarity between the jacketed receiver for vacuum distillation described by J. B. Cloke [IND. ENG. CHEM., Anal. Ed., 12, 329 (1940)], and a receiver designed by Dr. Linnell of the Pharmaceutical Society of Great Britain. The Linnell type, described in a bulletin of the manufacturer, differs mainly from that suggested by Cloke in that it is not graduated, has no "dripper", has a simplified stopcock arrangement, and has proportionately more condenser surface between the side entry and the vacuum connection. The receiver is a modification of the Perkin vacuum triangle. Both receivers may be used advantageously with the new jacketed Pyrex flasks made by the Corning Glass Co.

Rectification Column for a Chemical Engineering Laboratory

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SUITABLE equipment for the study of rectification in an undergraduate laboratory has been a serious problem in chemical engineering instruction for some time. Such equipment may be purchased from one of the many companies supplying distillation apparatus to the industry, or it may be built in the shops of the university itself.

If the still is purchased, it may be one of the stock small-scale models produced by several equipment manufacturers. However, most companies are more interested in the sale of large-scale equipment, and offer the universities smaller units only as a service or assistance to educational facilities. In many cases such equipment is unsatisfactory for instructional purposes, in that either it is usually not sufficiently flexible to permit the study of widely differing conditions of operation, or insufficient arrangements are provided for complete sampling of the liquid and vapor at all points in the column. Moreover, the construction is generally of such a nature that changes in the apparatus are difficult to make.

Satisfactory designs may be submitted to an equipment manufacturer for construction. This procedure will eliminate the shortcomings mentioned above, but the cost will usually be prohibitive. On the other hand, if the equipment is designed and constructed at the university, excellent results can be produced, and the cost may be kept very low, especially where a mechanic's services are available. Such a column has been recently described in the literature (*2*). In addition, a unit has been constructed at New York University which is sufficiently different to make it of interest to others confronted with the same problem. The total cost of the material, exclusive of the condenser, was approximately $200.

Design

The complete apparatus consists of a boiler, a column, and a condenser with the usual accessories. The design throughout is of such a nature as to provide all possible means for obtaining complete test data, to anticipate all possible variations in operating conditions, and to allow for easy dismantling and reconstruction. For example, the boiler is a unit in itself, and may be separated easily from the column. The heating surface is composed of several independent units, allowing large variation in the heat input. The column provides for investigating either simple batch or continuous operation with or without forward flow. Feed can enter at any plate, permitting even the extremes of running the column as a stripping or as an enriching section. Temperature measurements, rates of flow, and samples of both liquid and vapor can be obtained at all vital points. In addition, provision has been made for performing distillation with open steam, or under pressure or vacuum.

The column proper contains ten plates, built up as separate sections. Each section is made from a standard 6-inch steel

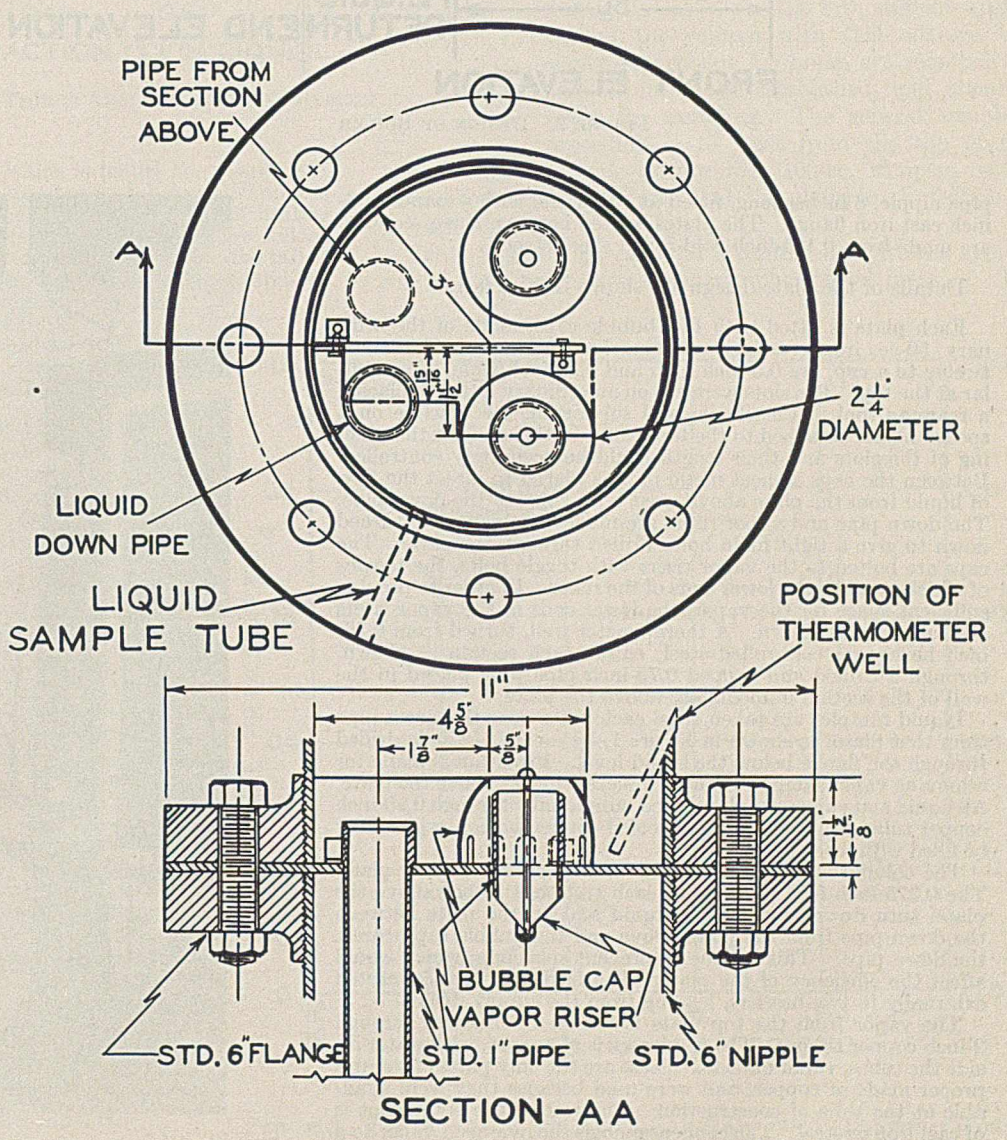

FIGURE 1. DETAIL OF PLATE

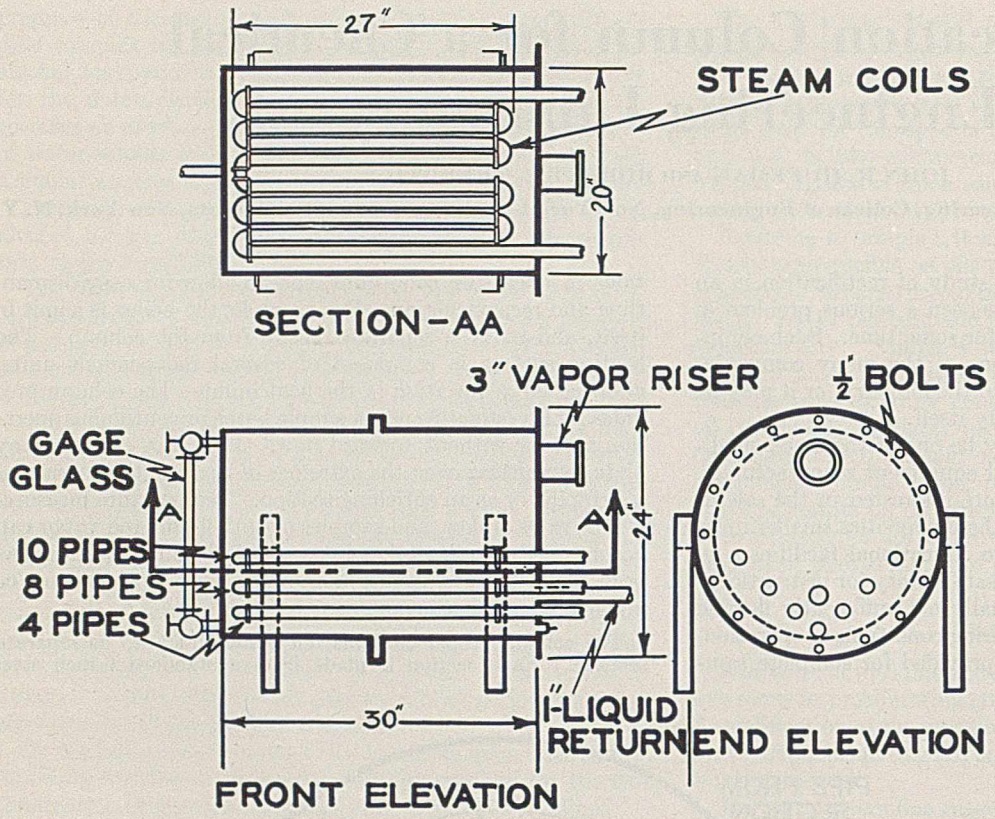

FIGURE 2. DESIGN OF BOILER

pipe nipple, 8 inches long, fitted at either end with a standard 6-inch cast iron flange. The plates, bolted between these sections, are made from 0.125-inch cold-rolled sheet steel.

Details of the plate design are shown in Figure 1.

Each plate is fitted with two bubble caps, made of the ordinary 50-cc. iron crucibles used in chemical work. The slots, twelve to a cap, are 0.5 inch high and 0.25 inch wide, semicircular at the top. The slots were cut on an ordinary lathe by placing a reaming tool in the chuck, and supporting the crucible on a special holder attached to the tool rest. In this manner the spacing of the slots and their length could be accurately controlled. Between the caps a sheet metal dam is placed to direct the flow of liquid from the plate above, past both caps, to the down pipe. The down pipe and vapor risers are made of 1-inch pipe, turned down to give a tight fit in holes drilled through the plate. The caps are bolted to the vapor risers with toggle bolts, the toggles of which straddle the lower ends of the risers. In order to provide sufficient space for the vapor, the lower ends of the vapor risers are cut away as shown. A thermometer well, turned from 0.56-inch hexagonal cold-rolled steel, enters each section as shown, through a drilled and tapped 0.75-inch pipe plug placed in the wall of the section immediately above the plate.

Liquid samples are taken from each plate near the down pipe from that plate, as shown in Figure 1, by means of a hole drilled through the flange below the liquid level. Provision is made for removing vapor samples from each plate 6 inches above the plate. All liquid and vapor samples are led individually through 0.25-inch copper tubing through a water-cooled condenser box, which may be filled with ice.

The column is designed so that feed may enter at any plate. The 0.375-inch feed pipes enter each section 4 inches above the plate, turn down, and form a liquid seal on the plate between the down pipe from the plate above and the bubble cap nearest the down pipe. This is done to prevent splashing, which would affect the efficiency of the plate. The feed pipes are connected externally to two headers, leading from the supply drum.

The vapor from the top plate is led to a condenser through 2-inch copper tubing. The condenser is of copper, with water inside the tubes, vapor outside. These are the only parts of the still proper made of copper, and were used because they were available at the time of construction. The rest of the equipment is of cast iron or steel. The condenser cools the overhead vapor to a cold liquid. The product may be withdrawn and the reflux returned through an orifice device for continuous rate measurement similar to one described by Zimmerman and Lavine (*2*). The cold reflux then passes through a short double-pipe steam-heated heat exchanger before entering the column at the top plate. It enters the plate in the same manner as the feed, at the point where the down pipe would normally come from the plate above. It is felt that this method of dealing with the problem of hot reflux is best, as experience with other student equipment has proved that difficulty is encountered with a condenser which removes only the latent heat from the overhead vapors. With the arrangement described, the reflux can be heated and kept without difficulty to within 1° F. of its boiling point.

Details of the boiler design are given in Figure 2.

The shell is made from 0.188-inch steel, rolled and welded into a cylinder 20 inches in diameter and 30 inches long. One end is closed by a plate welded to the cylinder. The other end has

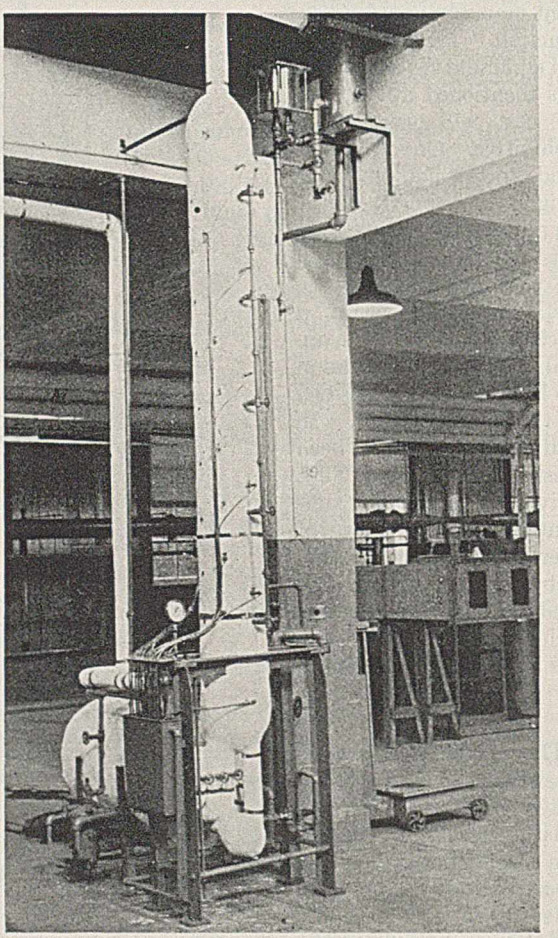

FIGURE 3. DISTILLATION UNIT

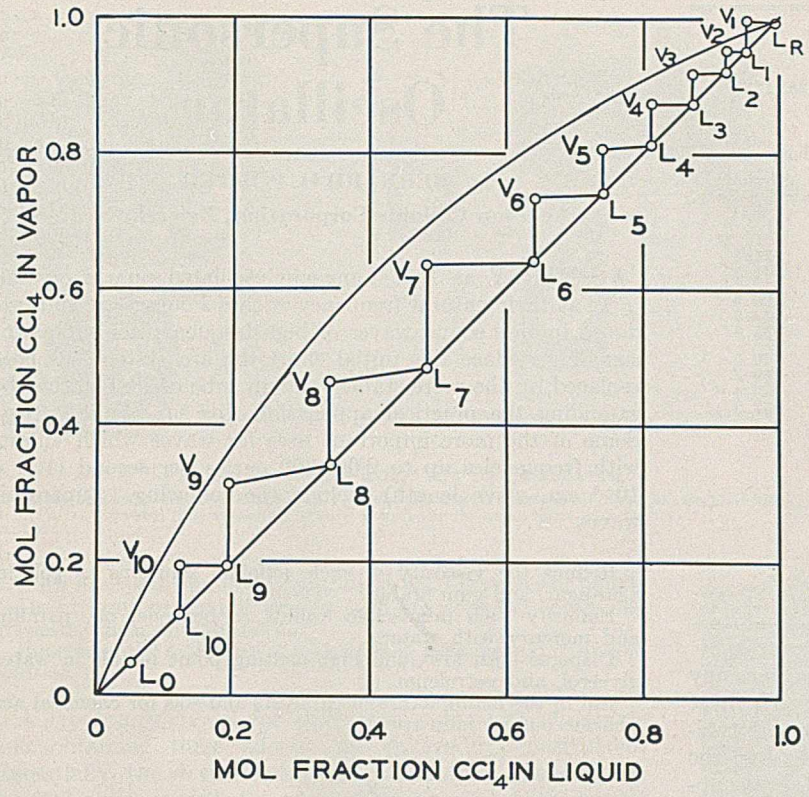

FIGURE 4. MCCABE-THIELE ANALYSIS AT TOTAL REFLUX

welded to it a 2-inch flange to which is bolted the cover plate. The entire unit is set up with its axis horizontal, mounted on four legs bolted to lugs welded to the sides. Three sets of heating coils, made of 1-inch pipe with return bend elbows, are welded to the cover plate. These extend into the lower half of the boiler. The top coil consists of ten pipes, the middle of eight, and the lower of four. Steam from a single header can be admitted to these coils singly or to any combination of them. Condensate from all three coils passes out through a simple Sarco thermostatic steam trap to a drain, with provisions for measurement.

Experience has shown that this design of heating surface works more satisfactorily than a jacketed kettle since, with the entire heating surface always submerged, no violent splashing of liquid occurs and superheating of vapor is avoided. In addition, the heating area may be varied at will. The vapor leaves the boiler through a 3-inch standard pipe leading to the bottom of the column. Liquid returns from the column by means of a 1-inch pipe through the cover plate, arranged with a liquid seal. Both pipes enter the column below the tenth plate into a section similar to the others. A 1-inch pipe is welded into the top of the boiler for charging and for inserting a long-stemmed Weston dial thermometer which extends below the liquid level. Gage glass fittings are welded to the rear plate. A 1-inch pipe leads from the bottom of the pot through a water cooler for withdrawal of bottoms. The design permits all connections to the column proper to be easily removed and reconstructed.

Asbestos sheeting was found to be a very satisfactory gasket material, and was used throughout. After assembly, the entire unit was insulated with 85 per cent magnesia pipe covering and magnesia cement.

Figure 3 is a photograph of the front of the assembled still. The positions of the sample lines, sample cooler, thermometer wells, valves to control steam flow to the heaters, liquid return, condenser, and reflux orifice, are evident. Table I lists other pertinent data.

TABLE I. MISCELLANEOUS DIMENSIONS

Distance between plates, inches	8.5
Plate area, sq. inches	28.891
Slot area per cap, sq. inches	1.199
Slot area per plate, sq. inches	2.398
Down pipe area, sq. inches	0.864
Vapor riser area, sq. inches	1.728
Heating surface, sq. feet	
Top coil	4.82
Middle coil	3.85
Bottom coil	1.93
Total	10.60
Condenser	
43 tubes, 0.635 inch outside diameter, length, inches	17.75
Area of tubes, sq. feet	10.4
(with tube sheets)	11.1

Operation

Carbon tetrachloride–toluene is the mixture used for student runs. This proves to be an ideal mixture for laboratory tests, since equilibrium characteristics show no abnormalities (*1*). Furthermore, analysis of samples is simplified, since the difference in densities of the two components is so great that a Westphal balance gives sufficiently accurate and rapid determinations of the composition. Experience during the last 6 months with student operation of the column with this mixture has shown that about 1.5 hours are required for equilibrium to be established, and about 2 hours for sampling. The general sampling procedure is to start from the top of the column and to work downward; 100-cc. samples, withdrawn sufficiently slowly so that the plates are not drained, are sufficient.

Table II and Figures 4 and 5 contain data from a typical run at total reflux and one atmosphere total pressure. No

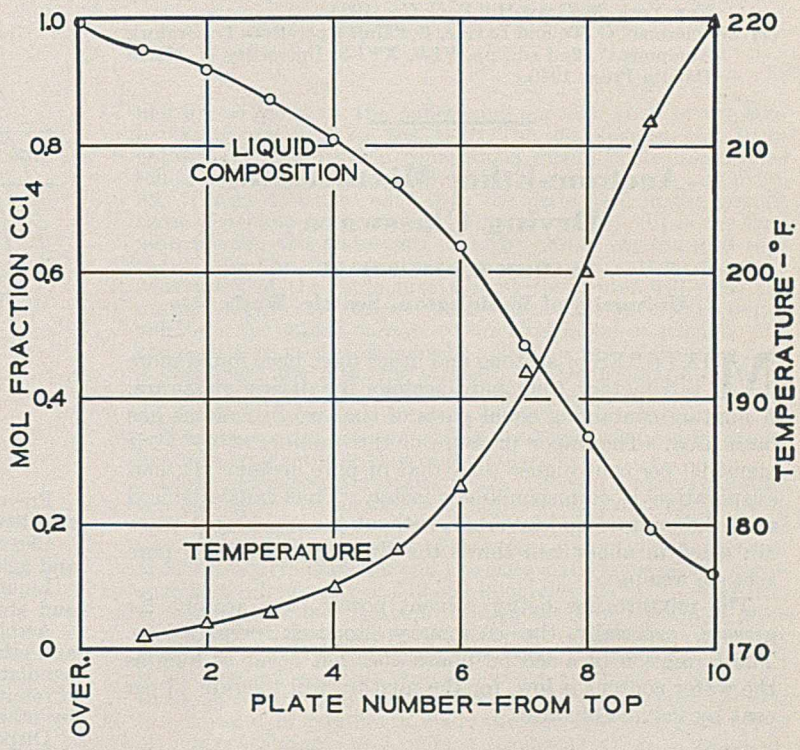

FIGURE 5. TEMPERATURES AND LIQUID COMPOSITIONS ON PLATES AT TOTAL REFLUX

TABLE II. DATA OF TYPICAL RUN

Conditions of operation: total reflux, atmospheric pressure
Mixture: carbon tetrachloride, b. p. 170.2° F.; toluene, b. p. 231.1° F.
Heaters used: middle and lower
Steam: 15 pounds per sq. inch gage; 21.48 pounds per hour
Reflux rate: 182 pounds per hour
Cooling water: in, 43° F.; out, 46° F.; rate, 6630 pounds per hour

Plate No. from Top	Temperature, ° F.	Composition, Mole Fraction CCl_4 Liquid	Vapor	Murphree Plate Efficiency, %
1	171	0.950	0.995	161.
2	172	0.920	0.952	71.1
3	173	0.873	0.916	61.5
4	175	0.811	0.873	60.8
5	178	0.740	0.805	49.2
6	183	0.640	0.732	55.0
7	192	0.483	0.632	70.9
8	200	0.340	0.463	59.7
9	212	0.191	0.314	76.4
10	220	0.121	0.192	57.3
Boiler	226	...	...	...
Reflux at orifice	56	0.992	...	...
Reflux into column	169	0.992	...	...
Liquid return to boiler (L_0)	..	0.050	...	...

Over-all plate efficiency, 78%
Heat balance: Given up by steam, 20,300 B. t. u. per hour
Taken out by cooling water, 19,900 B. t. u. per hour
Unaccounted for, 400 B. t. u. per hour, 1.97%

attempt was made during the run to maintain conditions any more carefully than is the practice with students. The heat balance is good, and the individual plate efficiencies are typical. These latter vary a little from plate to plate, and the particularly high efficiency of the top plate is not always obtained.

Acknowledgment

The authors wish to acknowledge the assistance of Wallace Pritchard, departmental mechanic, in the construction of the still, and Albert Capuro, of the department, for the drawings.

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Acetone-Ether Mixtures for Drying Glassware

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MIXTURES of acetone and ether have been found more satisfactory than pure acetone for drying glassware. A mixture containing equal parts of the two by volume has been used. The vapor pressure of this mixture at 30° C. is about 90 per cent higher than that of pure acetone (*1*), and evaporation is correspondingly faster. Thus bottles rinsed with the mixture and inverted, without the use of suction or air, dried in about two thirds the time required when pure acetone was used.

The mixture has better solvent power than acetone for greases, especially the customary stopcock preparations. The formation of a second phase does not occur so long as the water content is low, for the mixture will tolerate 13 per cent by volume of water.

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The Supersonic Oscillator

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AS EARLY as 1917 Langevin oscillated quartz crystals at their natural frequency with a Poulsen arc and produced intense sound waves of high frequency for submarine signaling. Since this initial effort the arc system has been replaced by the more stable vacuum tube oscillator, thereby extending the practical applications for supersonic energy. Some of the more important uses for waves which vibrate with frequencies up to 2,000,000 cycles per second (1.5×10^{-4} cm. wave length) include the following. Supersonic waves

Reduce the viscosity of such colloidal solutions as gelatin, agar-agar, and gum arabic
Emulsify such immiscible liquids as benzene, oil, paraffin, and mercury with water
Disperse both low- and high-melting point metals in water, glycerol, and petroleum
Aid in the manufacture of catalysts and sols for chemical and pharmaceutical industries

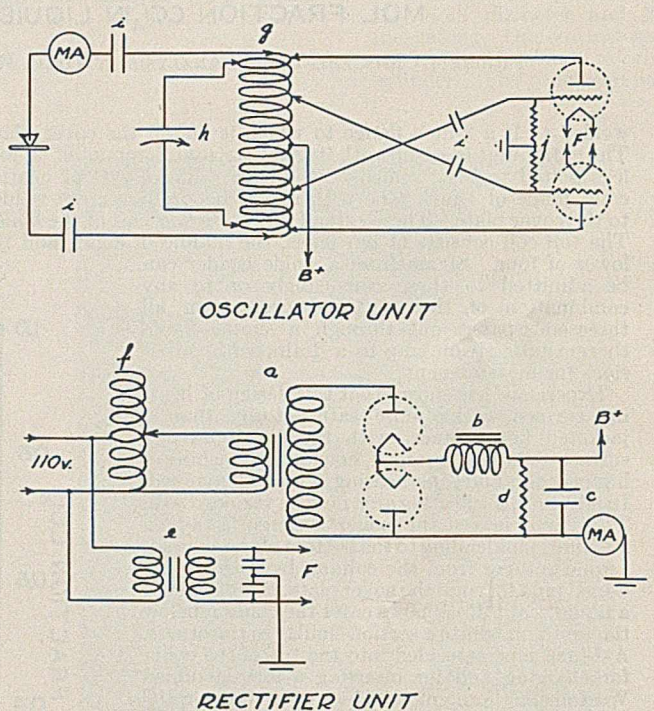

FIGURE 1. OSCILLATOR AND RECTIFIER

Prevent grain formation and improve homogeneity and stability during the preparation of photographic emulsions
Cleave such highly polymerized molecules as starch, gum arabic, and gelatin
Liquefy such thixotropic gels as iron hydroxide, bentonite, and aluminum hydroxide
Accelerate peptization (the rate of solution of rubber in aniline and ether, for example, is increased by this means. Supersonic irradiation also peptizes hydrated precipitates such as iron, chromium, and aluminum hydroxide, which cannot be reduced by other mechanical means)
Disperse liquids like benzene, toluene, and water in air
Coagulate aerosols
Orient in liquids such anisodimensional particles as mica, glass, and quartz

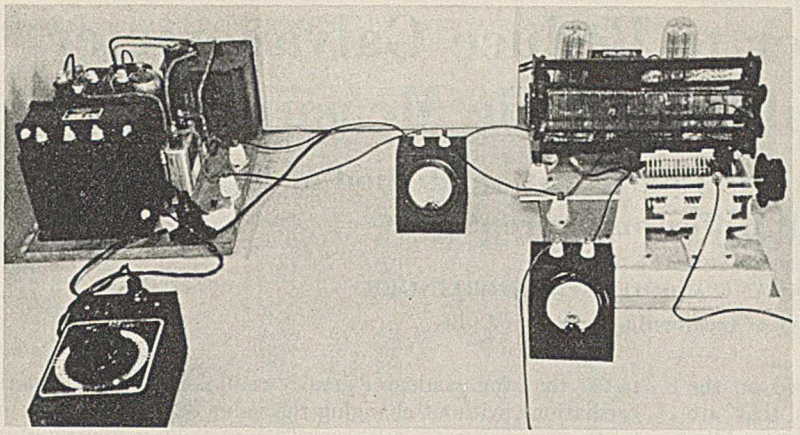

FIGURE 2. TABLE-TOP VIEW OF OSCILLATOR
Rectifier unit left; oscillator unit right. Leads running off illustration (at right) connect with crystal unit shown in Figure 4, and should be as short as possible.

The oscillator portion of the circuit consists of two No. 242C, 100-watt vacuum tubes (Western Electric), a tank coil, *g*, and tuning condenser, *h*, together with associated by-condensers, *i*, and grid leak resistors, *j*. A 0–0.5 high frequency ammeter records the oscillator output.

Insulator supports must be used wherever any of these parts would otherwise touch the mounting base. A distance of at least 60 cm. (2 feet) should also separate the two units, keeping the iron in the transformers of the rectifier unit away from the tank coil and tubes of the oscillator. The apparatus of each unit may be mounted on separate baseboards measuring 30 × 75 cm. (1 × 2.5 feet) (Figure 2).

The tank coil employed in the oscillator portion of the circuit requires special care in its construction. It consists of 88 turns of varnished copper wire (1.125 mm., 0.045 inch in diameter) on a 30-cm. (12-inch) long Bakelite frame, 15 cm. (6 inches) in diameter. The coil has eight adjustable taps, two pairs going to the plates and grids of the tubes and one pair each to the tuning condenser and to the cell. The distance in coil turns between the various tap connections should be maintained as follows: tuning condenser, 68; grid, 23; output, 85; and plate, 85. (These distances vary with the parts used in the circuit; those given appear to provide optimum results. If, in the final adjustments, the plate taps must be moved in, the grid taps should also be moved a corresponding percentage of turns. Movement of the plate taps toward the center will raise the output voltage; movement of the output taps toward the center will lower the output voltage. Care must be taken when moving the plate taps in that the output voltage is not raised to so high a point that the dielectric strength of the insulating media is broken down.) Taps connecting the tube plates and coil are on the same turn as the taps leading to the condenser. The grid taps are purposely crossed, in order that the grid output of one tube may activate that of the other.

Of all piezoelectric crystals, quartz is chosen for the activating cell because of its great mechanical strength and comparative ease of cutting along its optical and electrical axes. The crystal used with the circuit described measures approximately 3.44 cm. (1.375 inches) in diameter and possesses a thickness which corresponds to a frequency of 512.560 kilocycles (24° C.). One pole of electrical contact is made through the lead block and brass base on which the immersed crystal rests (Figure 3). The other electrode consists of concentric brass rings, the outer one of which is mounted on porcelain supports above the base. Connection to the inner ring which rests lightly on the crystal face and aids in holding it in position is made through four springlike strips of copper (Figure 4). Common insulating oil is a useful dielectric or crystal immersion fluid. The height of the fluid above the crystal face is carefully regulated to give an optimum fountain head.

Both flocculate and deflocculate suspended particles in liquids
Disintegrate or disrupt lamellar and low cohesion bodies like graphite, mica, and steatite
Rearrange the molecules of benzazide in benzene and in aniline solution

A laboratory model for producing supersonic vibrations and obtaining these effects can be readily constructed. Essentially, the device consists of two units, a high-voltage rectifier and an oscillator which motivates a quartz crystal immersed in a dielectric fluid.

The rectifier which supplies 1100 volts direct current to the oscillator utilizes two RCA 866 mercury vapor rectifying tubes, a high-voltage transformer, *a*, swinging choke, *b*, filter condenser, *c*, bleeder resistor, *d*, and filament transformer, *e* (Figure 1). The output voltage is controlled by a variable transformer, *f*, connected to the input of *a*. A continuous variation from 0 to 1100 volts is thus possible. The current supplied is measured by a 0–250 milliammeter.

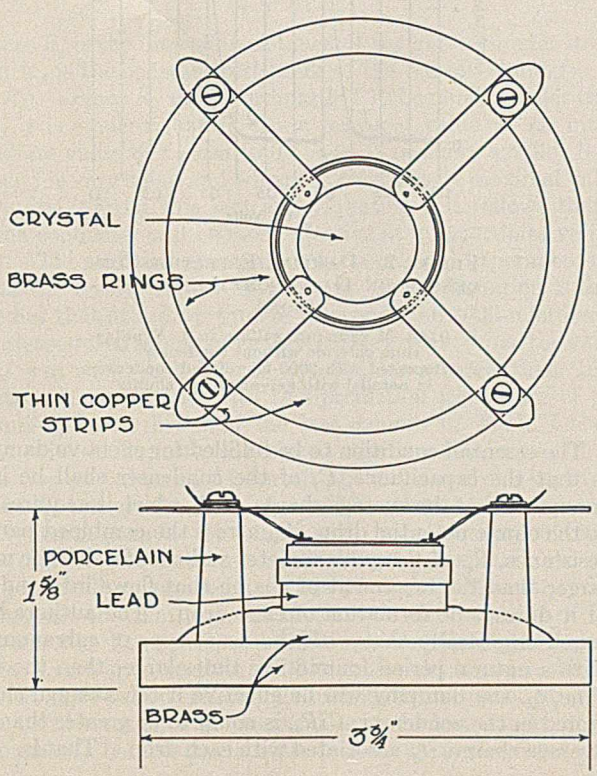

FIGURE 3. SPECIAL HOLDER

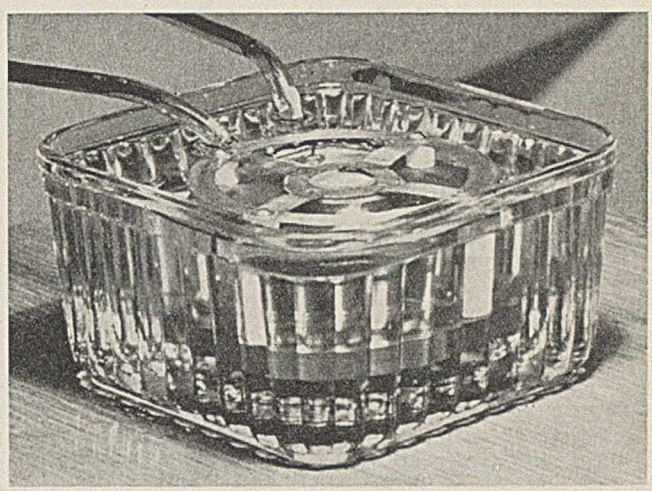

FIGURE 4. CRYSTAL UNIT

Use of a Condenser to Reduce Galvanometer Oscillations in Polarographic Measurements

With Particular Application to Compensation Method of Measuring Small Diffusion Currents

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THE polarographic method of analysis is based on the interpretation of the current-voltage curves that are obtained when solutions of electroreducible or electrooxidizable substances are electrolyzed with the aid of a dropping mercury electrode (*1, 2, 3*). As a result of the periodic change in area as each mercury drop grows and falls at the dropping electrode, the current varies from virtually zero at the very beginning of the formation of a drop to a maximum value at the instant the drop falls.

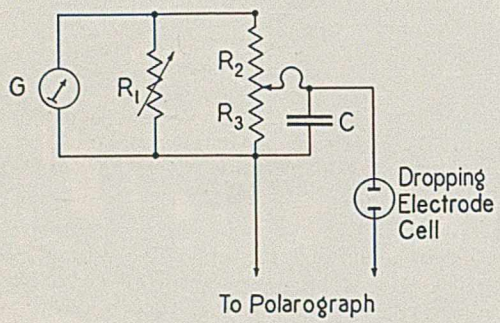

FIGURE 1. METHOD OF CONNECTING A CONDENSER, *C*, INTO CIRCUIT FOR DAMPING GALVANOMETER OSCILLATIONS

The average current is generally less than 100 microamperes, and it is usually measured by means of a sensitive mirror galvanometer of relatively long period (20 seconds or greater). With such an instrument the observed oscillations are much smaller than the true change in current during the life of each drop; they usually amount to about 5 to 10 per cent of the average current. The oscillations are very uniform, and ordinarily there is no great difficulty in measuring their average value, which corresponds very closely to the true average current (*3, 4*). However, in certain cases the oscillations are large enough to be troublesome and it is therefore very desirable to have a method of reducing their magnitude. This is particularly true in the "compensation method" of measuring small waves which is described below. The authors have found that the most satisfactory method of damping the oscillations without affecting the value of the diffusion current is to connect an electrolytic condenser of high capacitance across the galvanometer shunts as indicated by *C* in Figure 1. They employed a Heyrovsky-Shikata type of polarograph whose principle has been described elsewhere in detail (*1, 2, 3*).

The effect of the condenser is demonstrated by the typical current-voltage curves in Figure 2. Curve 1 in this polarogram was recorded in the usual manner (*1, 2, 3*) with an air-free solution of 0.001 *M* cadmium sulfate in 1 *N* potassium chloride, without a condenser and with a drop time of 4.2 seconds. Curve 2 was recorded with the condenser (2000 microfarads) in the circuit as shown in Figure 1. It will be noted that the condenser greatly reduced the galvanometer oscillations, without changing the value of the diffusion current.

The condenser damps the galvanometer oscillations by decreasing the variation in e. m. f. across the shunts of the galvanometer, that otherwise occurs as a result of the periodic change in current through the cell. When a mercury drop falls the condenser partially discharges, and more or less completely maintains constant current through the galvanometer until the next drop has grown sufficiently so that the current through the cell is restored to its average value, $\bar{i}$. During the later growth of the drop, when the current through the cell increases above its average value, the condenser absorbs an increment of charge equal to that lost by discharge when the preceding drop fell. Since the internal resistance of the electrolytic condenser is very large compared to that of the galvanometer and shunts, there is no net flow of current through it, and hence the average deflection of the galvanometer is the same with and without the condenser.

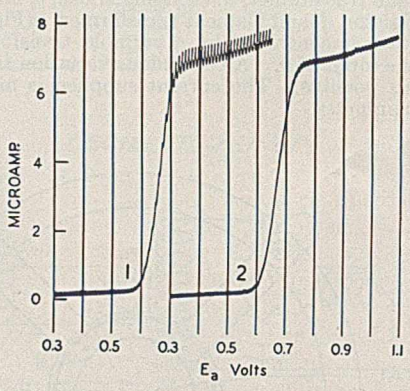

FIGURE 2. DAMPING EFFECT OF CONDENSER ON GALVANOMETER OSCILLATIONS

1. 0.001 *M* cadmium sulfate in 1 *N* potassium chloride without condenser
2. Repeated with 2000-microfarad condenser in parallel with galvanometer shunts

The essential condition to be fulfilled for effective damping is that the capacitance, *C*, of the condenser shall be large enough so that the average charge, $C\bar{i}R_s$, which it acquires due to the ohmic potential drop, $\bar{i}R_s$, across the combined parallel resistance, R_s, of the galvanometer and shunts, shall be much larger than the increment of charge that flows into and out of it during the formation of each drop. The authors have found empirically that with the usual type of galvanometer with a natural period four to five times larger than the drop time, t_d, the damping will be effective if the average charge stored in the condenser, $C\bar{i}R_s$, is equal to or greater than the average charge, $\bar{i}t_d$, associated with each drop. That is,

$$C\bar{i}R_s \geq \bar{i}t_d \qquad (1)$$

TABLE I. DAMPING EFFICIENCY OF CONDENSER AT VARIOUS SETTINGS OF AYRTON SHUNT

(Various concentrations of $CdSO_4$ in 1 N KCl plus 0.005% gelatin, with a 2000-microfarad electrolytic condenser in parallel with the galvanometer and shunts as shown in Figure 1. Drop time = 3 seconds; R_g = 38 ohms; R_1 = 7.7 ohms; R_A = 10,000 ohms; oscillations without and with the condenser compared at $E_{d.e.} = -1.0$ volt *vs.* S. C. E. Shunt setting f adjusted to give a total deflection of 90 to 110 mm. at each concentration of $CdSO_4$.)

$CdSO_4$ Millimolar	f	R_s Ohms	Oscillations Without condenser Mm.	Oscillations With condenser Mm.	Damping Factor
0.4	0.99	105	4.5	4.0	0.9
0.5	0.95	475	6.0	2.5	0.4
0.6	0.75	1875	6.0	0.7	0.12
0.7	0.70	2100	6.0	<0.5	<0.1
1.0	0.40	2400	4.5	<0.5	<0.1
2.0	0.20	1600	4.0	<0.5	<0.1
3.0	0.15	1275	7.0	1.0	0.14
5.0	0.09	820	7.0	2.0	0.28
10.0	0.045	430	8.0	3.5	0.44
25.0	0.020	200	7.0	5.0	0.7
50.0	0.010	100	7.5	7.0	0.9

or

$$C \geqq \frac{t_d}{R_s} \times 10^6 \text{ microfarads} \qquad (2)$$

when t_d is expressed in seconds and R_s in ohms. This equation is only an approximation, of course, but is a useful guide in choosing the proper capacitance for a particular circuit.

The value of R_s, and hence the required capacitance, varies with the setting of the Ayrton shunt (R_2R_3 in Figure 1). If we represent the setting of the Ayrton shunt—i. e., the fraction of the full sensitivity—by f, then it is evident from Figure 1 that

$$f = \frac{R_3}{R_2 + R_3} = \frac{R_3}{R_A} \qquad (3)$$

and

$$R_s = \frac{\left[\frac{R_gR_1}{R_g + R_1} + (1 - f)R_A\right]fR_A}{\frac{R_gR_1}{R_g + R_1} + R_A} \qquad (4)$$

where R_g is the internal resistance of the galvanometer itself, and R_A is the constant resistance of the Ayrton shunt ($= R_2 + R_3$). Since R_g and R_1 ordinarily will be much smaller than R_A, it is evident that R_s has a maximal value at an intermediate value of f close to 0.5, and it becomes smaller both when f is very small and when f approaches its maximal value of unity. From this fact and Equation 2 it follows that a given condenser will be most effective at intermediate values of f. The authors have verified this conclusion experimentally with the results shown in Table I. From Equation 2 they predict that at a drop time of 3 seconds the 2000-microfarad condenser used should have been most effective when the value of f was such that R_s was equal to or greater than 1500 ohms. In agreement with this prediction the values of the damping factor listed in the last column of Table I show that with this particular circuit the condenser was most effective at values of f between about 0.1 and 0.8—that is, when R_s was greater than about 1000 ohms.

By employing a condenser with a capacitance of 5000 microfarads the authors found that the oscillations could be practically completely eliminated under optimum conditions, and the damping was effective down to a value of f of about 0.03. It was impractical to use a condenser of capacitance much greater than 5000 microfarads, because the apparent half-period of the galvanometer became so large (about 70 seconds) that the response of the instrument lagged appreciably behind the increase in current on the rising part of a wave.

Improved Compensation Method of Measuring Diffusion Currents

Suppose that a certain reducible substance, B, is to be determined and that the solution also contains a more easily reducible substance, A, that is present in much larger concentration. In order to obtain the small wave of the minor constituent, B, on the polarogram it is necessary to employ a relatively small sensitivity of the recording galvanometer, so that the large wave of A is also obtained, and under these conditions the small wave of B, preceded by the larger wave of A, is too small for accurate measurement. This is demonstrated by curve 1 in Figure 3 which was obtained with a solution containing 0.005 M cadmium sulfate and only 5×10^{-4} M zinc sulfate in 1 N potassium chloride. The small wave of the zinc, preceded by the large wave of cadmium, is too small for accurate measurement.

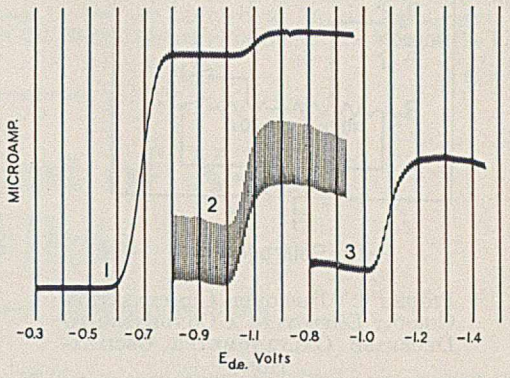

FIGURE 3. COMPENSATION METHOD OF MEASURING A SMALL DIFFUSION CURRENT PRECEDED BY A LARGE WAVE OF A MORE EASILY REDUCIBLE SUBSTANCE

In order to determine the minor constituent, B—e. g., Zn^{++}—without a preliminary chemical separation, various authors (*2*, *5*) have recommended that the interfering diffusion current of A—e. g., Cd^{++}—be compensated (reduced to zero) by sending a current of equal magnitude through the galvanometer in an opposite direction from an outside source. After the diffusion current of A is balanced out, the sensitivity of the galvanometer can be increased so that the wave of B becomes large enough for convenient measurement. This compensation method has heretofore not been very practical, and its application has been limited by the fact that the galvanometer oscillations retain about their same magnitude (in terms of microamperes) when the interfering diffusion current is balanced out, and when the sensitivity is increased to record the wave of the more difficultly reducible minor constituent the oscillations become so very large that they seriously interfere with the measurement. This difficulty can be eliminated, and the range of the compensation method increased manyfold, by using a condenser to damp the galvanometer oscillations as described above.

The compensation circuit shown in Figure 4 was employed in conjunction with a Heyrovsky-Shikata polarograph. The e. m. f. across the galvanometer and its shunts, generated by the interfering diffusion current, is balanced out by an opposing e. m. f. from an auxiliary 2-volt battery which is regulated by the rheostat, R_2, and the variable resistance, R_1.

The use of this compensation circuit is best explained by the typical example shown in Figure 3. After curve 1 was obtained in the usual way, the applied e. m. f. was set to 0.8 volt by manual adjustment of the bridge of the polarograph, so that the interfering diffusion current of the cadmium was obtained. Switch S_2 was then closed, and the compensating e. m. f. was adjusted by regulating R_1 and R_2 until the galvanometer deflection was reduced approximately to zero. The sensitivity of the

galvanometer was then increased by changing the setting of the Ayrton shunt from $f = 0.08$ to $f = 0.40$, a small readjustment of the compensating e. m. f. was made to bring the galvanometer deflection exactly to zero, and the wave of the zinc was recorded starting at an applied e. m. f. of 0.8 volt. Curve 2 was obtained after compensating the cadmium wave without the condenser in the circuit, whereas curve 3 was obtained with the condenser (2000 microfarads). Without the condenser (curve 2) the galvanometer oscillations are so large that the measurement of the wave height is difficult, but with the condenser (curve 3) the oscillations are greatly decreased and the wave height is easily measurable.

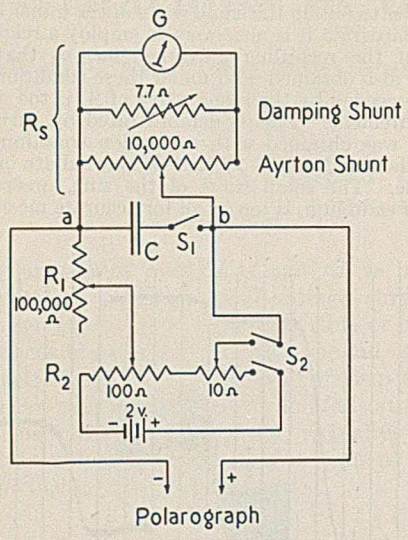

FIGURE 4. IMPROVED COMPENSATION CIRCUIT EMPLOYING A CONDENSER TO DECREASE GALVANOMETER OSCILLATIONS

C. 2000- to 5000-microfarad electrolytic condenser

Without the use of a condenser the compensation method is impractical when the concentration of the interfering major constituent is greater than about ten times that of the minor constituent, because of the exceedingly large oscillations of the galvanometer. On the other hand, when a condenser of the proper capacitance is used it is possible under optimum conditions to employ the compensation method up to a concentration ratio of the major and minor constituents of about fifty.

Any tendency of the diffusion current to change with changing applied e. m. f. is greatly magnified in the compensation method. The diffusion current usually tends to decrease with increasing negative potential beyond about −0.6 volts *vs.* the S. C. E. owing to the decrease of the drop time with increasing negative potential. With the ordinary method of measurement this change is usually not very noticeable (*3*) but it becomes very pronounced when the compensation method is used, as shown by curves 2 and 3 in Figure 3. This fact limits the application of the compensation method to very well-defined waves.

Summary

A method for decreasing the magnitude of the galvanometer oscillations in polarographic measurements uses an electrolytic condenser connected across the galvanometer shunts. By the proper choice of capacitance the galvanometer oscillations can be practically completely eliminated without affecting the diffusion current. The application of this principle greatly extends the practical applicability of the compensation method of measuring small diffusion currents. An improved compensation circuit is described.

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A High-Output Electric Flask Heater

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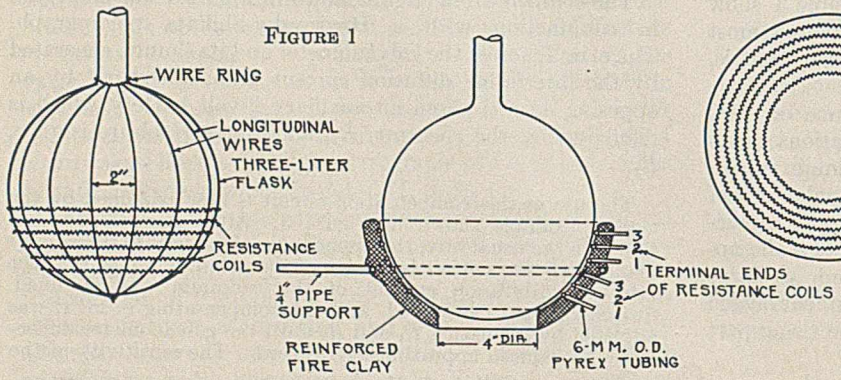
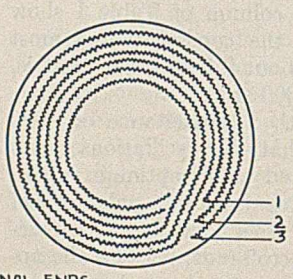
AN ELECTRIC flask heater was designed with sufficient heat output to distill higher boiling petroleum fractions (up to 343° C., 650° F.) and utilize maximum flask area for heat transfer. Its design permits supplementary heat with a Bunsen burner. Constructed with three separate heating units, it furnishes a wide range of heat output. It is simply made, inexpensive, and durable.

FIGURE 1



A 3-liter flask was used as a mold about which the heater was constructed. To support the heating coils during the application of the refractory material, a framework of wires was constructed about the flask. A ring of 16-gage wire was formed around the neck of the flask, and ten 30-cm. (12-inch) lengths of 26-gage wire were fastened at one end to the ring. The wires were evenly spaced about the neck of the flask and brought longitudinally to the bottom, where the ends were twisted together. Three heating coils were formed by winding separate 10.5-meter (35-foot) lengths of 20-gage oxidized Nichrome wire on an arbor 3 mm. (0.125 inch) in diameter. Each coil was slightly stretched, so that adjacent turns did not touch. The coils were placed parallel to each other on 1.56-cm. (0.625-inch) centers and wound about the flask as shown in Figure 1 (left and right). Thread was used to fasten the coils firmly to the longitudinal wires.



The heating coils, now firmly fastened to the flask, were entirely filled with softened paraffin wax. A thin layer of wax was then applied to the outside of the coils and to the area of the

flask upon which the refractory material was to be plastered (Figure 1, center and right). A conduit for the terminal ends of the resistance wire (Figure 1, center) was formed by 3.75-cm. (1.5-inch) lengths of 6-mm. Pyrex tubing.

Refractory material—Hitempite, Quigley Co., 56 West 45th St., New York, N. Y., a plastic used for binding firebrick in furnaces—was plastered over the assembly in four separate layers. Time was allowed after each application for drying, and between the second and third applications 1.56-mm. (0.067-inch) braided asbestos tubing was used for reinforcing material. The total thickness of the four layers of fire clay was approximately 3.1 cm. (1.25 inches).

The heating unit was removed from the flask by cutting the longitudinal wires and filling the flask with hot water to melt the paraffin. The wires still in place were removed after cutting the threads attaching them to the heating coils. The assembly was then placed under a hood, and a small current passed through each heating coil to volatilize the remaining paraffin and further dry the fire clay. (Care was taken to remove any small amounts of refractory material from the exposed side of the heating coils where they had become entirely encompassed in application.) When the wax had melted away, about two thirds of the outside diameter of each turn of the heating coil was surrounded by refractory material. Thus the coils were held firmly in the heater body.

A support was made for the heater from a length of 0.625-cm. (0.25-inch) pipe, bent into a circle of proper diameter to fit approximately the outer circumference of the heater at about half its horizontal height (Figure 1, center). One end was bent to extend laterally from the side of the heating unit and the pipe was cemented into place with sufficient refractory material to make it an integral part of the heater. A clamp can be used to support the heater on a ring stand.

Any desired heat output can be obtained within the maximum limits of the heater. Each resistance coil draws 4.5 amperes at 110 volts, giving approximately 500 watts maximum output per individual unit. The heating coils were arranged so that one, two, or three could be connected into a single lead.

The heater also operates conveniently with a 2-liter flask. A Bunsen burner can be used to furnish supplementary heat through the 10-cm. (4-inch) opening in the base of the heater.

Accurate Measurement of X-Ray Diffraction Films

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THE accurate measurement of line positions on powder and rotation photographs is a problem in all precision diffraction studies. This problem is complicated by the fact that x-ray lines cannot be subjected to high magnification, since they then tend to fade into the background. The usual types of comparators with high magnification are therefore unsatisfactory.

Numerous devices for measuring x-ray films have been described (*1–5*), and all of them are based on the use of a highly accurate engraved scale or an accurate micrometer screw. The chief problem is to set the movable pointer or cross hair accurately on the edge or center of the line. To accomplish this some have incorporated costly auxiliary equipment such as photometers (*1*), cathode-ray oscillographs (*5*), etc. The average device for these measurements is thus very costly.

The instrument described involves only minor changes in design, but it has proved to be simple to manipulate, sufficiently accurate, satisfactory for both powder and rotation photographs, and economical to construct, since it requires only standard available parts and simple machine work. The design of the movable pointer, means for holding the films in place, and source of illumination are improved features.

Apparatus

Figure 1 shows a line drawing of the complete measuring device. The base is a wooden case, *A*, with sloping front. A hinged back on the case gives ready access to the source of illumination mounted therein. The light source is a Day Brite fluorescent lamp unit (Westinghouse catalog, No. 8930) with a 2.5 × 45 cm. (1 × 18 inch), 15-watt, daylight Mazda lamp. A piece of milky glass placed between it and the film support results in a very

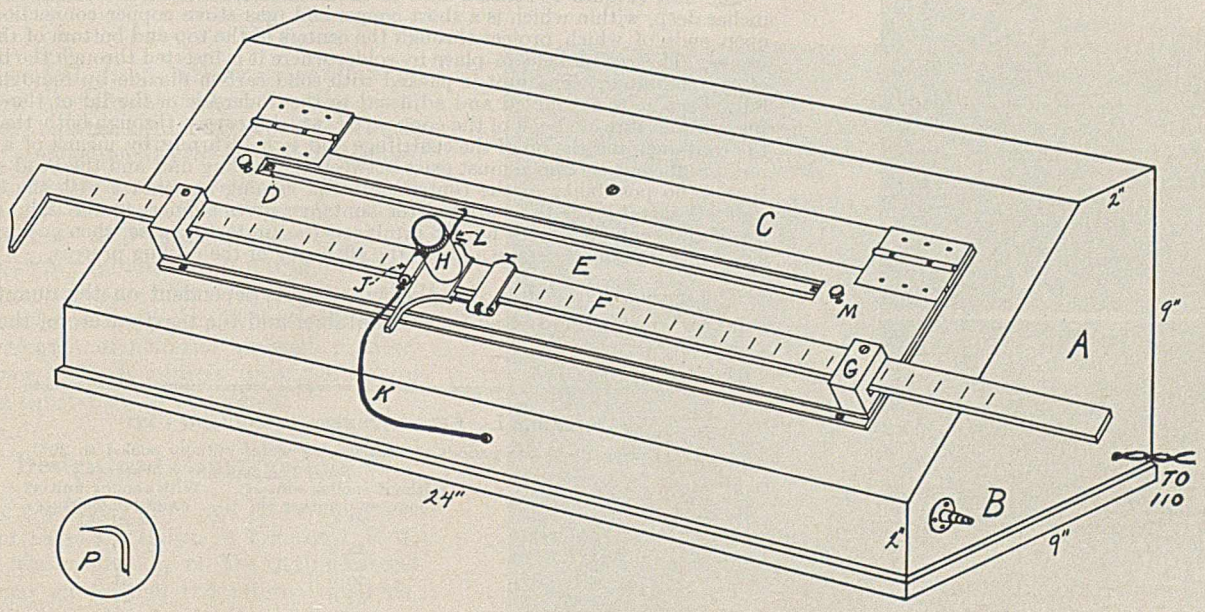

FIGURE 1. MEASURING DEVICE

uniform and satisfactory source of illumination. A cheaper, but less satisfactory, light source is a row of "eight in series" white Mazda Christmas-tree lamps behind the milky glass diffusing screen.

Particularly with the Christmas-tree lamps, and to a less extent with the fluorescent lamp, some cooling is desirable to prevent the film, scale, etc., from becoming warm and expanding during the course of a series of measurements. Such cooling is achieved by blowing compressed air through a series of holes in a brass tube, *B*, running the length of the case. Air holes in the back of the case permit circulation of the cooling air.

C is an aluminum alloy (17ST) plate, 46.45 × 12.5 × 0.469 cm. (18.5 × 5 × 0.188 inches), attached to the wooden case at three points. It has a 2.5 × 35 cm. (1 × 14 inch) opening directly over an illuminated slot in the wooden case. A piece of plate glass with its upper surface flush with the surface of *C* fills this opening, and serves as a support for 2.5 × 32.5 cm. (1 × 13 inch) powder films which are temporarily held in place by the spring clips *D*, at each end. *E* is a similar aluminum alloy plate, 46.45 × 8.75 cm. (18.5 × 3.5 inches), hinged to *C* at the upper edge. A 1.25 × 35 cm. (0.5 × 14 inch) beveled slot in it is centered over the opening in *C*. *E* is lifted up for insertion of a film and returned to position. When the film has finally been adjusted to the exact position for measurement it is clamped tightly in position between plates *C* and *E* by the removable thumbscrews, *M*, which are threaded into *C*. Plates *C* and *E* must be flat and have good surfaces. To ensure this it is well to specify that they be cut by sawing rather than by shearing.

The scale, *F*, is a 60-cm. vernier caliper (No. 122 M, L. S. Starrett Co., Athol, Mass.) graduated in 0.5-mm. divisions and provided with a vernier reading to 0.02 mm. It is clamped in position 1.875 cm. (0.75 inch) above plate *E* by the slotted blocks, *G*, fastened to *E*. Clamped to the movable jaw is a 2.5-cm. (1-inch) 4 × magnifying lens, in a tubular focusing mount, *H*, for reading the vernier. The vernier is illuminated by a 2.5-volt flashlight bulb in tube *J*. One contact is made at the end of *J* by a flexible wire, *K*, and the other through the metal parts of the instrument. A toggle switch on the left end of the case controls the light whose current source can be two dry cells or a small transformer. A rectangular lens would give a slightly better field in reading the vernier.

The movable jaw was also drilled at *L* and provided with setscrews for holding a pointer, *P*, in place. In Figure 1 is shown a detail of *P*, which is used in the horizontal position. The angle of the point should not be too sharp nor too broad—approximately 15° has been found satisfactory. The tip of the pointer should not end in too sharp a point but should be slightly dull. The pointer is adjusted to be just the thickness of a thin sheet of paper above the film, thereby preventing parallax errors. With this type of pointer and a low-power reading glass it is possible to set on the edge or center of a line with ease and accuracy. For example, in determining the position of the undeviated beam image on a powder film a single setting was made on the center of each of five pairs of lines symmetrically situated with respect to the exit beam. These measurements gave for the position of the beam image 36.308, 36.305, 36.306, 36.305, and 36.307; average, 36.306.

Rotation films are inserted in the device and shifted to bring successive layer lines into position for measuring.

Acknowledgment

The author takes pleasure in acknowledging the helpful suggestions and excellent workmanship of Floyd O. Grapp, instrument maker, in the construction of this instrument.

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A Centrifuge Cooling Unit

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SOMETIMES it is desirable to keep liquids 5° to 10° below room temperature during centrifuging. For those lacking a refrigerating unit an attachable cooling unit utilizing solid carbon dioxide may prove satisfactory. Such a unit was devised in this laboratory for the centrifuge (International Equipment Company, size 1, type SB centrifuge).

The unit (Figure 1) consists of a metal container, 9 inches in diameter and 2.5 inches deep, within which is a short copper coil (gas stove copper connection) the open ends of which project through the centers of the top and bottom of the container. The coil is held in place by solder where it is inserted through the bottom of the container. The unit is packed with solid carbon dioxide by removing the lid. Then it is assembled and adjusted to the underside of the lid of the centrifuge by the threaded end of the copper coil, which projects through both the lid of the container and the lid of the centrifuge and is held firmly by means of a metal nut. Sufficient clearance must exist between the cooling unit and the head so that there is no possibility of the centrifuge tubes coming in contact with the cooling unit. A metal brace to fit around the container gives support to the unit. If the lid of the centrifuge does not fit firmly on the protecting case when enclosed, insulating with adhesive tape adds to the efficiency of the cooling process.

The temperature attained in the centrifuge is dependent on the quantity of solid carbon dioxide placed in the container and the temperature of the laboratory.

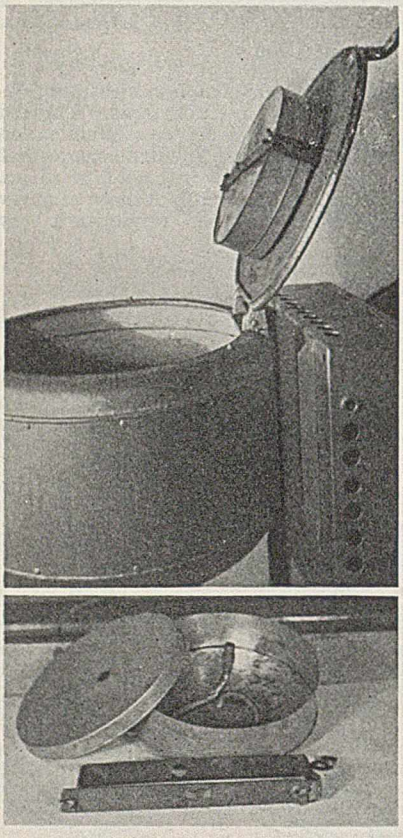

FIGURE 1. COOLING UNIT
Above. Adjusted to centrifuge lid
Below. Disassembled

TABLE I. EFFECTIVENESS OF COOLING UNIT

(During 10-minute periods of centrifuging water initially cooled to 20°)

R. p. m.	Final Temperature of Water: Without cooling unit at room temperature of 28° C.	With cooling unit at room temperature of 29° C.
1100	28.0	20.5
1800	29.0	20.0
2400	30.0	21.5
2800	32.0	21.5

A Laboratory-Size Leaf-Type Pressure Filter

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THE need of a small closed filter for laboratory operations was indicated during the studies of pilot-plant scale (*1*) and semicontinuous (*3*) production of gluconic acid by fermentation of glucose solutions. For the purpose of repeated noncontaminated recovery of mycelia for reuse in mold fermentations, several conditions must be met. The filter must be easily and positively sterilized, prevent the ingress of contaminating organisms, facilitate the return of mycelia to the fermenter, and be constructed of materials which do not inhibit further activity of the fermenting organism.

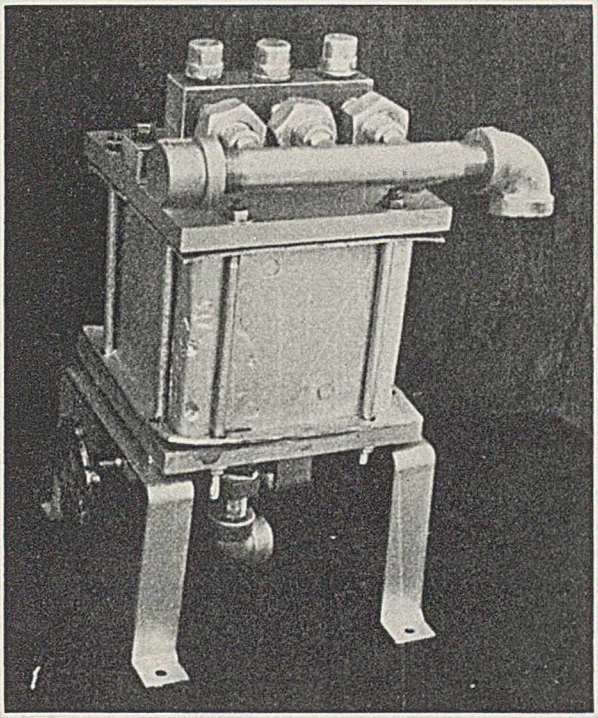

FIGURE 1. ASSEMBLED FILTER READY FOR OPERATION

In general, commercial pressure filters of the leaf type are satisfactory for pilot-plant studies, but the relatively large size of such units is objectionable for laboratory studies where comparatively small quantities of solids are handled. Therefore, for use in conjunction with fermentation studies in small, rotary, aluminum drums (*2*), a small leaf-type pressure filter was designed and constructed of aluminum at this laboratory. The selection of material for such a filter, however, depends entirely upon the nature of the substances to be filtered.

Design and Construction

The assembled filter and its various component parts are shown in Figures 1 to 4. With the exception of the stainless-steel machine screws and the external tie rods, the filter assembly was fabricated from commercially pure aluminum alloys.

The filter case consists of a 11.25-cm. (4.5-inch) square tubular section and top and bottom closing plates [1.25 cm. (0.5 inch) thick and 15 cm. (6 inches) square]. The tubular section was made from four plates, each 0.625 × 11.25 × 12.5 cm. (0.25 × 4.5 × 5 inches). The short sides of these plates were mitered and welded together with the same metal by means of atomic hydrogen. The top and bottom plates were held in place by eight steel tie rods threaded through the bottom plate and secured by lock nuts. Rubber gaskets at top and bottom prevented leakage.

Three filter-leaf assemblies (Figures 3 and 4) adaptable for either top or bottom drainage provided the actual filtering elements. The square leaf frames with 7.5-cm. (3-inch) openings were 1.875 cm. (0.75 inch) thick. The inner edges of the frame were rounded to prevent cutting of the filter cloths. In the top of the frame a hole was drilled and tapped from both ends for $^1/_8$-inch aluminum tubing to provide means for carrying away the filtrate. (Dimensions with reference to tubing sizes are I. P. S.) The section of tubing within the frame (Figure 3) was removable so that top drainage might also be accomplished. Each frame was covered with woven aluminum filter cloth held in place by aluminum retainers, or frame covers, 0.156 cm. (0.0625 inch) thick, the shape of which corresponded to that of the leaf frames. Stainless-steel machine screws and nuts securely held the frame covers and filter cloths to the frames.

A header block and manifold assembly provided means for consolidation and discharge of the filtrate. This block was constructed from a piece of 2.5 × 5 cm. (1 × 2 inch) aluminum bar stock 15 cm. (6 inches) long, fitted with a thin rubber gasket, and bolted to the top plate as shown in Figure 4. Three holes were drilled vertically through the top plate and header block to accommodate the discharge tubes from the filter leaves. Three discharge ports were drilled on one side of the block to meet the vertical holes, and were tapped for $^1/_8$-inch pipe connections to the manifold. The small size of this filter unit necessitated displacement of the end discharge ports to allow sufficient clearance for 0.31-cm. (0.125-inch) aluminum unions. The discharge tubes from the top of the filter leaves extended through the vertical holes in the header block and were secured at the upper ends by 0.31-cm. (0.125-inch) aluminum pipe caps. Leakage of the filtrate was prevented by means of rubber gaskets between the top plate and filter leaves and by a combination of rubber gaskets and aluminum washers between the pipe caps and header block. A 0.625-cm. (0.25-inch) hole, drilled in the discharge tube at the position of the discharge port in the header block, permitted the filtrate to pass from the tubes to the manifold. Short aluminum nipples and unions connected the header block to the manifold. Three holes were drilled and tapped for $^1/_8$-inch tubing in a section of $^1/_2$-inch aluminum pipe in positions corresponding to

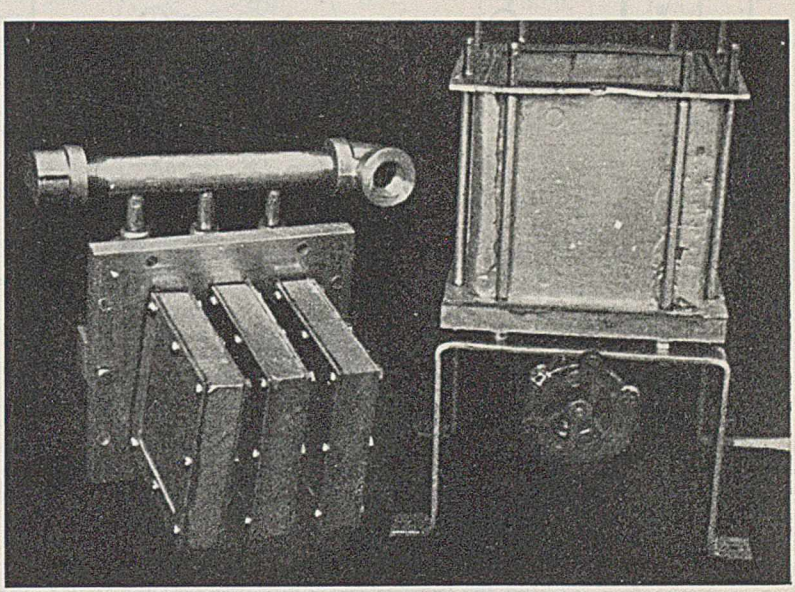

FIGURE 2. FILTER CASE AND TOP ASSEMBLY, SHOWING FILTER LEAVES

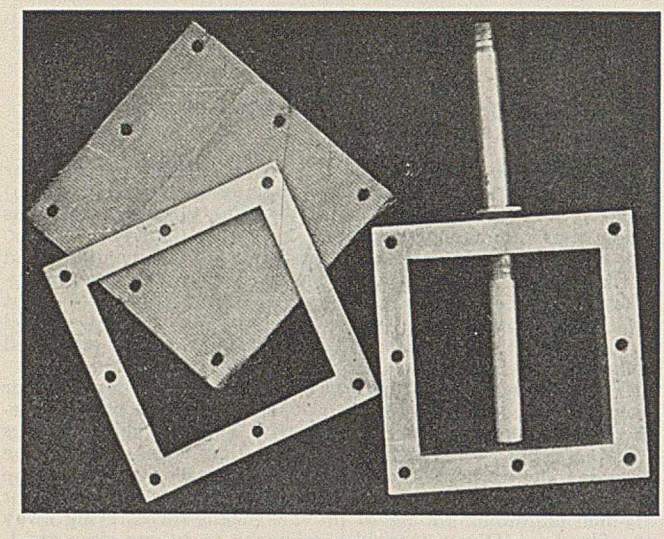

FIGURE 3. COMPONENTS OF FILTER LEAF, SHOWING ONE FILTER CLOTH AND FRAME COVER

those of the discharge ports in the header block. This section was threaded at both ends and served as the discharge manifold.

The inlet port, a hole in the center of the bottom plate, was tapped for $^3/_8$-inch pipe. In order to provide space for valves and fittings beneath the filter, two supports were formed from steel straps (Figure 1) and fastened to the lower ends of the tie rods.

Discussion

The assembled filter has a calculated, wet-cake (solids) capacity of 0.69 liter. The internal hydraulic pressure to which this filter is usually subjected is 30 pounds per square inch (3.16 kg. per sq. cm.), the pressure used in the fermentation studies for the semicontinuous production of gluconic acid (*3*). The effective filtering surface of this unit is 54 square inches (0.348 square meter).

A filter of such design can be constructed without difficulty in any laboratory equipped with the usual shop facilities. The present unit is satisfactory. However, the construction could be improved by: welding the bottom plate to the square tubular section; welding the header block to the top plate; welding a flange section around the top of the square tube to permit the use of short bolts instead of long tie rods;

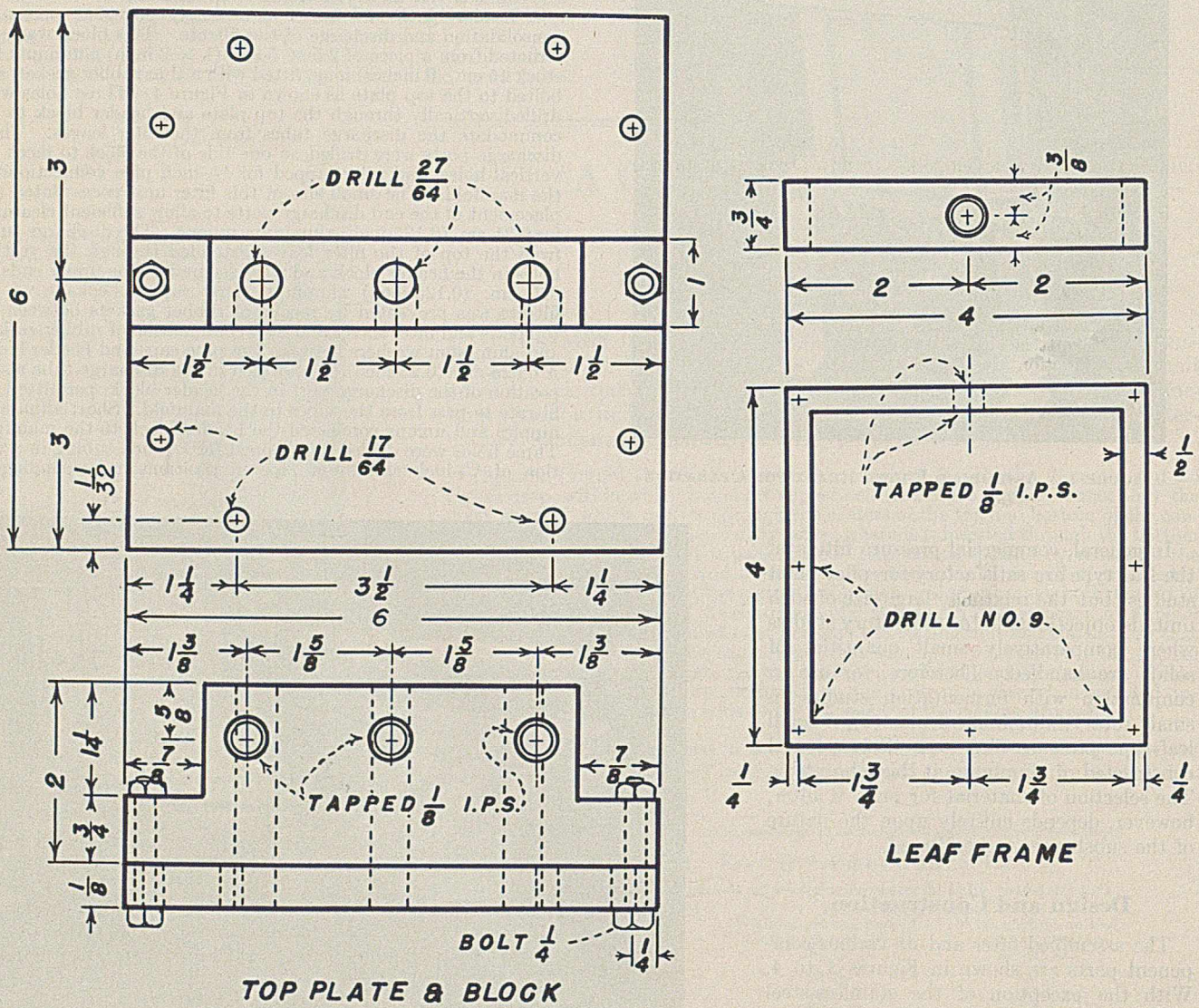

FIGURE 4. DETAILS OF TOP PLATE, HEADER BLOCK ASSEMBLY, AND LEAF FRAME FOR FILTER

All dimensions in inches

and installing a vent in the top plate to permit the exhaustion of air entrapped when bottom drainage is employed.

The principles upon which this filter operates are not new. In 1912, a patent on a pressure leaf filter in which the basic principles are similar to that described in this manuscript was issued to Sweetland (*4*). Later he was granted a patent for a bottom drainage leaf of circular design (*5*). However, to the authors' knowledge, this is the first time that square leaves and the choice of either top or bottom drainage have been incorporated into a single, laboratory-size filter assembly of simple construction.

Summary

A laboratory-size, leaf-type pressure filter has been designed and constructed for experimental studies. Simple construction permits fabrication in the average laboratory shop. The filter leaves are adaptable to either top or bottom drainage.

This filter has an effective filtering surface of 54 square inches and a calculated, wet-cake capacity of 0.69 liter. Hydraulic pressures up to 30 pounds per square inch (gage) have been used successfully.

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(1) Gastrock, E. A., Porges, N., Wells, P. A., and Moyer, A. J., IND. ENG. CHEM., 30, 782–9 (1938).
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(3) Porges, N., Clark, T. F., and Gastrock, E. A., *Ibid.*, 32, 107–11 (1940).
(4) Sweetland, E. J., U. S. Patent 1,032,091 (July 9, 1912); reissue No. 14,213 (Nov. 7, 1916).
(5) Sweetland, E. J., U. S. Patent 1,381,944 (June 21, 1921).

AGRICULTURAL By-Products Laboratory established by the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, in cooperation with the Iowa State College.

An Electronic Relay

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THE control of the electrical power requirements of thermostats, barostats, and similar equipment by electronic relays is common practice. A large number of circuits have been described in this journal and a list of the more recent literature references has been given by Waddle and Saeman (*1*). In all these circuits hot-cathode, high-vacuum tubes have been employed. However, in the past year radio tube manufacturers have developed, and now stock as a standard item, a cold-cathode, starter anode, gas-filled tube, Type OA4G, which is particularly suitable for relay purposes.

A standard circuit utilizing this tube is shown in Figure 1, together with a pictorial diagram of the octal base socket as viewed from the top.

In this circuit any size of radio potentiometer may be used, as only 0.25 watt is dissipated in the potentiometer. A relay which will function on 25 milliamperes will be suitable, as this current is the maximum permissible current for the normal operation of the OA4G tube. In practice, 110-volt (3000 ohms) and 32-volt (1000 ohms) relays have been found very satisfactory. For low-resistance relays sufficient additional resistance should be connected in series with the relay so that the total direct current resistance in the relay circuit is about 1500 ohms. The wattage dissipated in this series resistor is so small that ordinary 1-watt radio resistors may be used for this purpose.

The condenser, C_1, eliminates chattering of the relay caused by the pulsating direct current passing through the tube. Its capacity should be kept as low as possible. The 2-mfd. value listed in Figure 1 is suitable for the 3000-ohm relay. Relays of lower resistance will need a higher capacity; the 1000-ohm relay requires 4 mfd.

The resistance, R_2, tends to diminish sputtering of the electrodes and thus increases the life of the tube. The value of R_2 is not critical and 500-ohm to 3000-ohm resistors have been used.

To adjust the relay, the movable contact arm of the potentiometer is placed somewhere near the middle of its range. The regulator contacts are shorted and the relay is connected to the 110-volt alternating current line. The contact arm of the potentiometer is then moved until the tube glows and the relay closes. The arm should be moved in such a direction that R_1' is greater than R_1''. On proper adjustment, R_1' will be about 33,000 ohms. No attempt should be made to adjust the potentiometer exactly to that point at which the tube just begins to glow, as some allowance must be made for variations in the line voltage.

As one side of the alternating current line is usually grounded, accidental grounding of the regulator leads should be avoided. If such a possibility exists, a 20,000-ohm resistance should be placed in one of the control leads.

A cold-cathode, gas-filled tube has the following advantages over a hot-cathode, high-vacuum tube: Properly operated, the cold-cathode tube will have the longer life. With the regulator contacts open, the cold-cathode electronic relay dissipates 0.25 watt, while the hot-cathode relays, which generally use resistors in the filament circuit to drop the voltage to the proper value, will dissipate from 6 to 33 watts, depending on the type of the tube employed. With a 110-volt source, a gas-filled tube gives a much higher plate current than can be obtained from high vacuum tubes and thus cheaper and more rugged relays may be employed. With the cold-cathode tube, no current passes through the relay when the regulator contacts are open and consequently there is no tendency for the relay armature to stick.

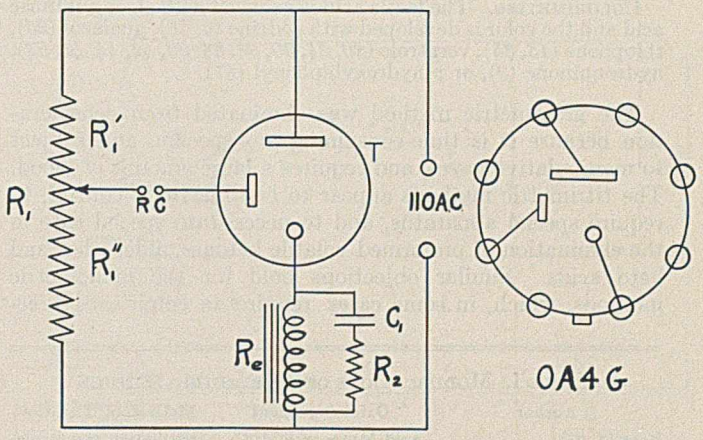


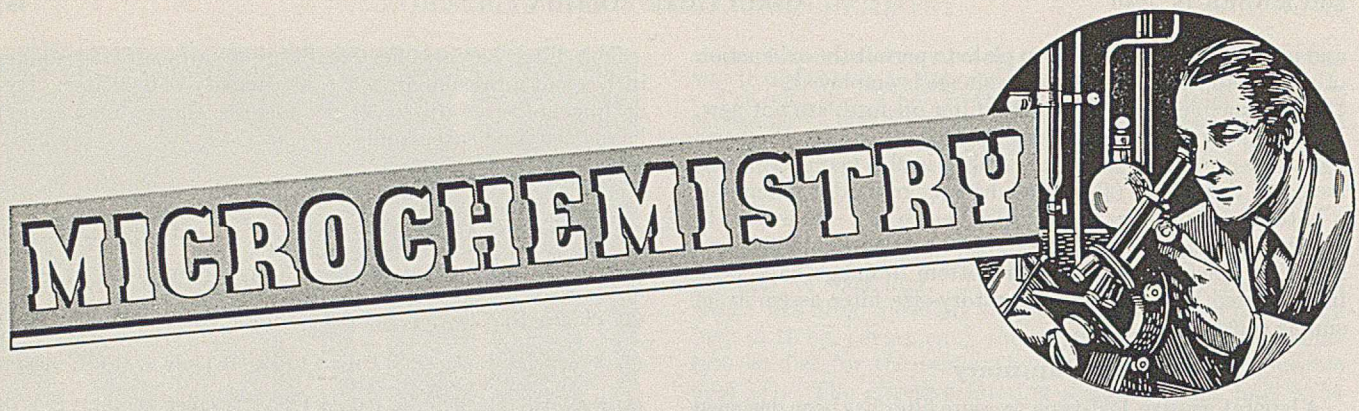
FIGURE 1. STANDARD CIRCUIT

R_1. 50,000-ohm potentiometer
R_2. 2000-ohm resistor, 2 watts
C_1. 2-mfd. condenser
T. Cold cathode, gas-filled triode, Type OA4G
RC. Regulator contacts
R_e. Relay, Utah, Type RAC-110

All essential parts for the construction of this relay can be obtained from any radio supply house. The total cost of standard parts including tube, relay, and sockets is less than $4.

Literature Cited

(1) Waddle, H. M., and Saeman, W., IND. ENG. CHEM., Anal. Ed., 12, 225 (1940).



Determination of Lactic Acid in Blood

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Duke University School of Medicine, Durham, N. C.

THE numerous modifications of existing methods for the determination of lactic acid in blood are evidence of the inadequacy of any one method. It is for this reason that, prior to undertaking a clinical investigation requiring the determination of blood lactic acid, the literature on the methods was reviewed and an attempt to standardize a single method was made.

Choice of Methods

Four basic procedures were considered.

GRAVIMETRIC (*13, 15, 19, 31, 57*). This method depends upon the isolation and weighing of partially hydrated zinc lactate.

TITRIMETRIC. The lactic acid is oxidized to acetaldehyde which is determined titrimetrically. Modifications of this method are listed in Table I.

MANOMETRIC. The carbon dioxide evolved during the oxidation of lactic acid by permanganate in concentrated acid solutions (*1, 2*) or the carbon monoxide produced by the action of concentrated sulfuric acid alone (*37, 48*) is measured.

COLORIMETRIC. The lactic acid is oxidized with strong sulfuric acid and the color is developed with codeine (*6, 50*), guaiacol (*25*), thiophene (*13, 35*), veratrole (*20, 24, 30, 32, 38, 39, 42, 44, 47, 54*), hydroquinone (*9*), or *p*-hydroxybiphenyl (*41*).

The gravimetric method was eliminated from consideration because it is time-consuming, nonspecific, and subject to manipulative losses, and requires a large amount of blood. The titrimetric methods appear to be difficult of control, to require special apparatus, and to necessitate special care in the elimination of preformed volatile ketones, aldehydes and keto acids. Similar objections hold for the manometric methods, which, in some cases require an empirical correction for unknown substances in order to obtain agreement with the titrimetric method. Of the colorimetric methods, the Mendel-Goldscheider (veratrole) seemed to have been investigated more fully than the others. The literature indicated the advantages of specificity, simplicity, high sensitivity, and the ability to perform a large number of determinations simultaneously. This method was therefore adopted for standardization. In order to facilitate the reading of the color, the use of the Evelyn photoelectric colorimeter (*12*) was incorporated in the method.

TABLE I. MODIFICATIONS OF TITRIMETRIC METHOD

Author	Oxidizing Agent	Method of Titration
Boas (*3, 27*)	Acid MnO_2 or $KMnO_4$	Distillation and formation of iodoform. Titration of excess iodine
Furth and Charnass (*21*)	Acid $KMnO_4$	Distillation into standard bisulfite and determination of excess bisulfite iodometrically
Clausen (*4, 7, 10, 33, 34, 36*)	Acid $KMnO_4$	Distillation and determination of bisulfite binding power
Friedemann *et al.* (*11, 15–18, 26, 48, 55, 56*)	Acid $KMnO_4$ plus $MnSO_4$	Distillation or aeration and determination of bisulfite binding power
Jervell (*28, 29*)	Acid $K_2Cr_2O_7$	Determination of excess dichromate
Gordon and Quastel (*22*)	Acid $Ce(SO_4)_2$	Distillation and bisulfite binding

Reagents

Ten per cent aqueous trichloroacetic acid, freshly prepared each month and stored in amber bottles in the ice box.

Fifteen gram per cent aqueous solution of copper sulfate.

Finely powdered calcium hydroxide. Ingvarrson (*26*) has indicated that some samples of calcium hydroxide give high values. Only samples giving negligible blanks should be used. If the blank is high, the calcium hydroxide can be purified by washing with water, drying at 104° C., and pulverizing in a mortar.

Concentrated sulfuric acid, standardized and stored as indicated below.

Veratrole, 0.125 per cent in absolute ethanol.

Standard solution of lithium lactate (106.3 mg. per 100 ml. ≙ 100 mg. per cent lactic acid).

Procedure

PRECIPITATION OF BLOOD PROTEINS. Blood (1.0 ml. collected with the usual precautions in a bottle containing 10 mg. of ammonium fluoride per 5 ml. of blood) is added to 7.0 ml. of distilled water in a 50-ml. Erlenmeyer flask. Smaller quantities of blood may be used, decreasing the amounts of other reagents proportionately. After hemolysis is complete, 7.0 ml. of trichloroacetic acid are added slowly and with vigorous mixing. The flasks are stoppered (rubber), shaken vigorously, and allowed to stand 20 minutes. The mixture is placed in a 15-ml. tube and centrifuged at moderate speed for 5 minutes.

REMOVAL OF INTERFERING SUBSTANCES. To another centrifuge tube, 4.0 ml. of the supernatant fluid are transferred, and 1.0 ml. of copper sulfate solution and approximately 1 gram of calcium hydroxide are added. The tubes are stoppered (rubber), shaken vigorously at intervals during a period of at least 30 minutes, and then centrifuged at high speed for 10 minutes. Filtration cannot be used, as filter paper contains substances which react as lactic acid (*55*).

OXIDATION OF LACTIC ACID AND DEVELOPMENT OF COLOR. Avoiding the surface film, a quantity of the supernatant fluid (the exact amount determined as under Standardization of Sulfuric Acid) is transferred to the bottom of a meticulously clean and dry Pyrex test tube (22 × 175 mm.) standardized for the 520 filter of the Evelyn colorimeter. To similar tubes is added the same quantity of water containing 0.5, 10, 20, and 30 micrograms of lactic acid as lithium lactate. If determinations are performed frequently it is not necessary to run a set of standards each time, since an average standard curve may be used for each bottle of sulfuric acid. All tubes are placed in an ice bath for 10 minutes.

TABLE II. RECOVERY OF LACTIC ACID FROM BLOOD

Author	Pptg. Agent	Final Concn. of Pptg. Agent	Dilution of Blood	Recovery from Standard Soln.	Recovery of Lactate Added to Blood	Method
		%		%	%	
Mendel and Goldscheider (*39*)	HPO_3[a]	0.63	1–8	...	95–98	Colorimetric
Ingvarrson (*26*)	HPO_3	0.63	1–8	96.2	87	Colorimetric
	HPO_3	0.63	1–8	89.1	98 (corrected for low value of standard)	Titrimetric
Dische and Rand (*10*)	HPO_3	0.63	1–8	...	95.7	Titrimetric
Ørskov (*45*)	HPO_3	0.63	1–8	...	86 (80–90)	Titrimetric
	Na_2SO_4 + H_2SO_4	and ether extraction	1–6	...	97.6 (95–102)	Titrimetric
	H_2WoO_4	1.0	1–10	...	83 (76–91)	Titrimetric
Ronzoni and Wallen-Lawrence (*48*)	HPO_3	1.0	1–10	...	95	Titrimetric
	H_2WoO_4	2.0	1–5	98	83 (calcd.)	Titrimetric
	H_2WoO_4	1.0	1–10	98	94.5 ± 3	Titrimetric
	H_2WoO_4	0.5	1–20	98	96 (calcd.)	Titrimetric
Jervell (*28*)	H_2WoO_4	1.0	1–10	99.6	96.4	Titrimetric
Lauersen and Wahlländer (*33*)	H_2WoO_4	1.0	1–10	89	96–99	Titrimetric
Clausen (*7*)	H_2WoO_4	1.0	1–10	98 ± 3	93.5 and 99.0	Titrimetric
Wierzuchowski and Sekuracki (*56*)	H_2WoO_4	1.0	1–10	95	90	Titrimetric
Friedemann *et al.* (*16*)	H_2WoO_4	1.0	1–10	90–105	Not given	Titrimetric
Friedemann and Kendall (*18*)	H_2WoO_4	1.0	1–10	97–99	Not given	Titrimetric
Friedemann and Graeser (*17*)	H_2WoO_4	1.0	1–10	99 ± 0.5	Not given	Titrimetric
Authors' method (18 determinations)	Trichloroacetic acid	4.7	1–15	97–101	Maximum 101 Minimum 94 Average 97.4	

[a] Milton (*42*) finds that metaphosphoric acid interferes with the colorimetric method and thus cannot be used.

To each tube are added 6.0 ml. of cold concentrated standardized sulfuric acid; the standardized pipet (see Formation of Acetaldehyde) is used and the acid is allowed to run down the side of the tube which is shaken constantly in an ice-water bath. A precipitate (calcium sulfate) may form which will dissolve later. The contents of the tubes are mixed thoroughly, and the tubes are stoppered loosely (rubber) and immersed to two thirds of their length in a vigorously boiling water bath. Five minutes later, the tubes are removed and placed again in an ice bath for 2 or more minutes. (The precipitate mentioned above has dissolved.) Then 0.1 ml. of veratrole solution is added to each tube. The tubes are mixed, tilting so as to rinse the walls, restoppered, and replaced in the ice bath for 75 minutes. They are remixed at 30 and 60 minutes. Each tube is removed separately, quickly wiped dry, and read in the Evelyn colorimeter (520 filter) against the blank without lactic acid. The L values (relative extinctions) are obtained and the amount of lactic acid in the aliquot taken for analysis is read from the curve of the standards.

$$\text{Mg. per cent in blood} = \text{micrograms in aliquot} \times \frac{75}{40y}$$

where y is the quantity of supernatant solution added.

Precipitation of Proteins

The literature contains discrepancies in the reported recoveries of lactic acid from blood using identical or different methods of protein precipitation. Papers which give the recovery of lactic acid in standards and when added to whole blood are listed in Table II. The average recoveries vary from 86 to 98 per cent with metaphosphoric acid, and from 83 to 96 per cent with tungstic acid. On the other hand, recovery from standards is usually very good. Since the precipitation of the blood proteins is the most likely cause of these discrepancies (*43*, *45*), this phase of the procedure was examined carefully.

With each method of protein precipitation, recovery is a function of the dilution of the blood. Mendel and Goldscheider (*39*) showed that greater recoveries were obtained with 1 to 8 dilution than with a 1 to 5 dilution of blood using metaphosphoric acid. Ronzoni and Wallen-Lawrence (*48*) obtained similar results with tungstic acid in dilutions of blood up to 1 to 20, while Edwards (*11*) found that the lactic acid value of the same blood at a 1 to 10 dilution was only 81 per cent of that at a 1 to 50 dilution (tungstic acid precipitation). Using serum good recoveries are possible at relatively low dilutions. Edwards (*11*) obtained maximal values of lactic acid in serum at a 1 to 20 dilution and Miller (*41*) reported 102 to 104 per cent recovery of lactate added to serum using a 1 to 10 dilution with metaphosphoric acid.

The authors were unable to obtain more than 88.5 per cent recovery of the total lactate after the addition of lactate to blood if the blood were precipitated with freshly prepared solutions of metaphosphoric acid (final concentration from 0.5 to 2.0 gram per cent) in spite of dilution of the blood up to 1 to 15. Results with the zinc hydroxide method of Somogyi (*51*) varied from 73 to 84 per cent recovery with an average of 79 per cent. Initially the Folin-Wu precipitation yielded an average recovery of 90.4 per cent with variations from 79 to 104 per cent in different experiments.

The variation in recovery which occurred in the Folin-Wu precipitation method may be a function of the pH of the filtrate (*53*). When the reagents are prepared according to the methods of Folin (*14*) and Haden (*23*), the authors found that the pH of the blood filtrates varied from 2.9 to 5.0 (glass electrode). Both extremes of pH have been advised as the optimal for the general use of this method of precipitation (*14*, *23*, *40*, *46*). In order to determine whether the pH of the filtrate had any effect on the recovery of lactic acid, experiments were performed in which the recovery of total lactate, the volume of the precipitates, the pH, the clarity, and the presence of proteins (biuret test) in the supernatant were determined when the blood was precipitated in 1 to 15 dilution.

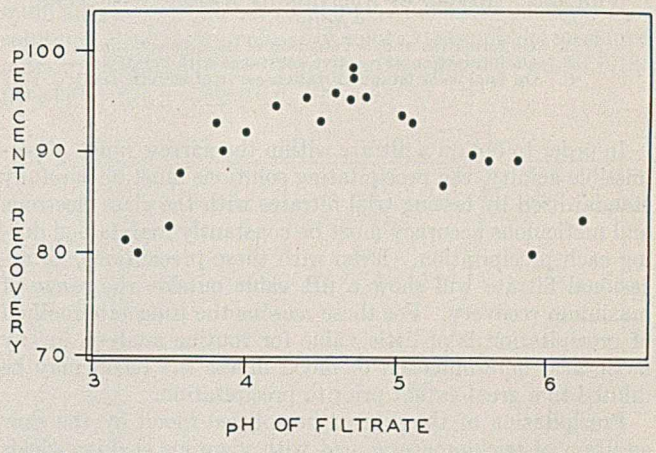

FIGURE 1. RECOVERY OF LACTIC ACID, ADDED TO BLOOD, AS A FUNCTION OF pH OF FOLIN-WU FILTRATE

Control experiments in which lactic acid was added to blood filtrates of varying pH always showed good recovery. These controls show that the pH of the filtrate does not influence the colorimetric portion of the test, but actually determines a loss of lactic acid during the protein precipitation. Thus the conclusions presented are applicable to all methods using tungstic acid precipitation of blood.

The percentage recovery of lactic acid is a function of the pH of the tungstic acid filtrates (Figure 1). The values are

at a maximum between pH 4.7 to 4.9, where the average recovery is 97 ± 2 per cent.

This dependency of recovery on the pH of the tungstate precipitation may explain the wide range of recoveries reported by different authors and the inconsistencies of the method noted by others (Table II) using different methods of analysis. The fortuitous occurrence of the correct pH may explain the statement of Edwards, "We have done some experiments with whole blood in which a 1 to 10 dilution gave the same results as 1 to 50, but we do not understand the cause of these variations from experiment to experiment" (*11*).

It is interesting to note that the volume of the precipitate reaches a maximum at pH 4.7. Filtrates below pH 3.4 and above pH 5.3 are cloudy; thus, the clarity of the filtrate is no indication that one will obtain adequate recovery of lactic acid. All filtrates above pH 5.3 give a positive biuret test.

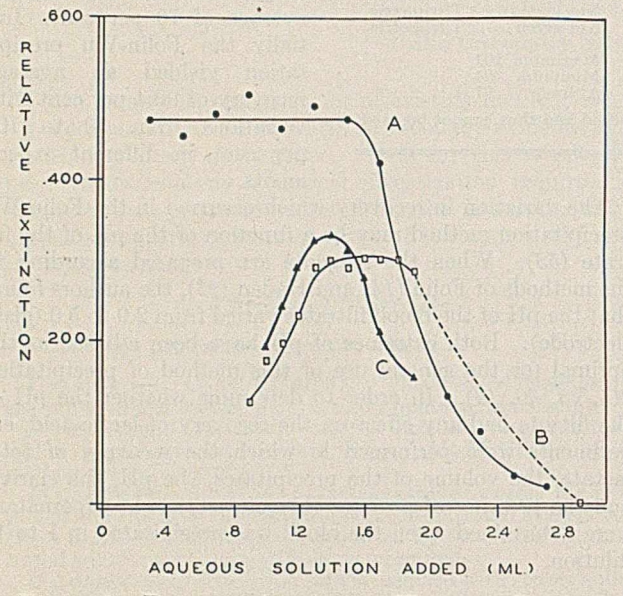

FIGURE 2. EFFECT OF ADDITION OF WATER TO SULFURIC ACID

A. On formation and/or retention of reactive substance
B. On interaction of reactive substance with veratrole
C. On final color (standardization curve of sulfuric acid)

In order to obtain a filtrate within the narrow range of permissible acidity, the precipitating solutions must be carefully standardized by testing trial filtrates with the glass electrode and meticulous accuracy must be constantly maintained during each precipitation. Even with these precautions, an occasional filtrate will show a pH value outside the range of maximum recovery. For these reasons the tungstate method of precipitation is of little value for routine analysis in any lactic acid determination of blood unless the blood may be diluted to a great extent prior to precipitation.

Precipitation of the diluted hemolyzed blood by the slow addition of trichloroacetic acid with vigorous shaking yields almost complete recovery of lactic acid (97.4 per cent, standard deviation = 2.4, Table II). If the same final concentration of acid is obtained by adding a more dilute trichloroacetic acid to the unhemolyzed undiluted blood, marked discrepancies between the results on successive samples of the same specimen are found. Similar discrepancies occur if the blood is added slowly to the dilute trichloroacetic acid. Apparently large protein masses form which occlude lactic acid in variable amounts. When the correct procedure is followed, the pH of the filtrate is approximately 1.5. Maximal recovery is thus possible at a different pH with trichloroacetic acid than with tungstic acid precipitation.

Removal of Interfering Substances

Few of the substances (sugars, pyruvic acid, paraldehyde, formaldehyde, acetaldehyde) which give colored compounds in this method are found in sufficient quantities in blood to interfere. Denigès (*8*) pointed out that some substances, yielding aldehydes on treatment with concentrated sulfuric acid, do so only when heated at much higher temperatures than that of boiling water. Glucose and other carbohydrates are completely removed by the treatment with copper sulfate and calcium hydroxide (*49, 52*). Pyruvic acid is eliminated to a great extent (*5*). Paraldehyde is not removed and will cause errors if present.

Formation of Acetaldehyde

Mendel and Goldscheider (*39*) showed that at least 4 minutes in the boiling water bath were necessary for the maximum conversion of lactic acid to acetaldehyde and that heating for periods up to 8 minutes caused no change in the yield. The authors have found, with lactic acid, a rapid increase in the reactive substance for the first 4 minutes. It then remains constant from 4 to 10 minutes of heating. Acetaldehyde, presumably the substance into which the lactic acid is converted (*8*), gives maximum color reaction without heating. On heating up to 10 minutes the maximum color developed (later) remains constant. Both acetaldehyde and lactic acid give decreasing color as the period of heating is prolonged beyond 10 minutes. Because of these observations the period of heating in the test was set at 5 minutes. The time is not critical; heating from 4 to 10 minutes gives the same results.

It is essential that temperature should not rise during the addition of sulfuric acid to the sample. The following precautions should be taken:

First, both sample and acid should be chilled in an ice bath before mixing; secondly, the first half of the acid should be added down the side of the tube at a rate not exceeding 1 ml. per 10 seconds (the remaining acid may be added as rapidly as desired); thirdly, the tube should be immersed in an ice bath and shaken constantly during the addition. If the first half of the acid is added too rapidly or if the acid is not adequately cooled, vapor forms and losses ensue. The appearance of vapor means that the sample must be discarded. The acid may be easily controlled by means of a slow-delivery pipet, to the upper end of which is sealed a glass stopcock.

In addition to the amount of heating and the method of sulfuric acid addition, the final color produced by a given amount of lactic acid is also a function of the concentration of sulfuric acid (*8, 32, 39, 54*). Milton (*42*) found it necessary to prepare special anhydrous acid and then add a definite quantity of solution to be tested.

Standardization of Sulfuric Acid

The authors have found it essential to standardize each bottle of sulfuric acid for its water content. The standardization is done as follows:

Into each of a series of tubes is pipetted 0.8 ml. of a standard solution containing 50 micrograms of lactic acid per ml. Enough distilled water is added to each tube to bring the total volume to 0.9 ml. in tube 1, 1.0 ml. in tube 2, and so on until 1.8 ml. is reached. In another tube 1.3 ml. of water alone is placed; this serves as the blank. Six milliliters of the new acid are added to each tube, observing the precautions outlined above. The tubes are heated and cooled, veratrole is added, and the color is developed and read as previously indicated. The L values are plotted against the total volume of water (lactic acid solution plus added water) in each tube. A curve is obtained (Figure 2, *C*) which shows a maximum usually between 1.2 and 1.5 ml. This maximum indicates the quantity of aqueous solution to be used in each determination with each 6.0 ml. of concentrated sulfuric acid from that bottle, which should be protected by a series of drying tubes.

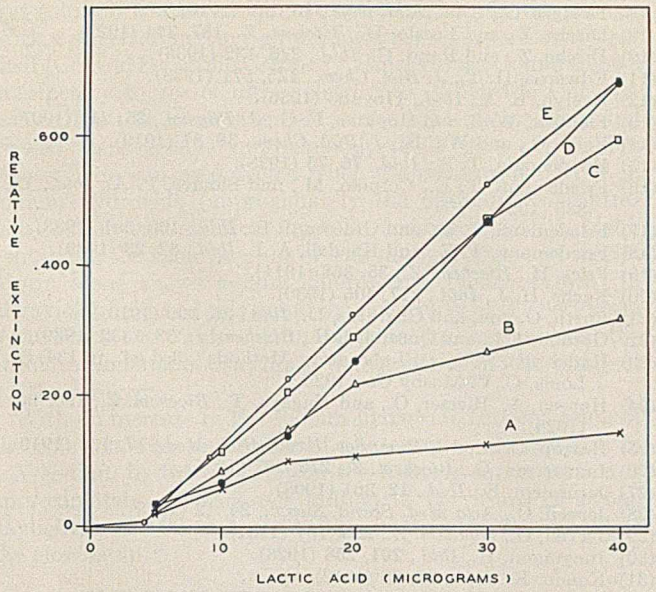

FIGURE 3. EFFECT OF INCREASING CONCENTRATION OF VERATROLE ON COLOR GIVEN BY DIFFERENT AMOUNTS OF LACTIC ACID

A. 0.2 ml. of 0.015 % veratrole
B. 0.2 ml. of 0.031 % veratrole
C. 0.2 ml. of 0.062 % veratrole
D. 0.2 ml. of 0.125 % veratrole
E. 0.1 ml. of 0.125 % veratrole

The literature has numerous references (*20*, *39*) to the failure of certain batches of sulfuric acid to give the proper color. The authors have tested several different brands (Merck's reagent, Merck's C. P., and Baker's C. P. analyzed) and have found them to be very satisfactory. The only difference was a variation in the optimum quantity of aqueous solution to be added (apparently as a result of slight differences in water content of each acid).

The effect of water on the final color developed may enter into two phases of the procedure: into the production of the active substance from lactic acid and its retention in the solution during the heating, and into the development of color through the interaction of the active substance and veratrole. The influence of the concentration of the sulfuric acid on the production and retention of the active substance may be tested by using the same quantity of lactate in different volumes of water, then adding the usual 6 ml. of sulfuric acid and heating. After heating, the sulfuric acid is brought back to the optimum concentration by adding either water or sulfuric acid. The volume of veratrole is adjusted to the volume of total solution. The *L* values are corrected for the dilution. The results are plotted in Figure 2, *A*. It is seen that the concentration of sulfuric acid down to a certain percentage (corresponding to the addition of 1.5 ml. of water to 6 ml. of concentrated acid) has no effect on the yield. With increasing dilution beyond that point there is a sharp drop due to failure in either production or retention of the reactive substance

The effect of the concentration of sulfuric acid on the development of color through the interaction of the reactive substance and veratrole may be tested by adding increasing amounts of water just prior to the addition of veratrole. The *L* values are again corrected for the dilution (Figure 2, *B*).

In summary, the standardization curve (Figure 2, *C*) is a composite of these two effects. With increasing concentration of acid from that point giving maximum color, the falling off is due to the effect on the reaction of veratrole with the reactive substance, whereas with decreasing concentration of acid from the optimum, the final color is limited by the formation or retention of the reactive substance during the heating stage. It is evident that each batch of acid must be standardized in the manner previously outlined in order that the sensitivity shall not be limited by either reaction mentioned above, and that the procedure shall not be carried out on either steep portion of the curve where small changes in the concentration of acid markedly affect the final color.

Color Development

In the final stage, the development of color for a given amount of reactive substance is a function of the amount of veratrole added, the quantity of alcohol added as veratrole solution, the temperature at which the color is developed, and the time period of color development.

AMOUNT OF VERATROLE ADDED. The effect of increasing amounts of veratrole in the same volume of alcohol is shown in Figure 3. With all amounts of veratrole tested, the curves relating extinction to concentration are S-shaped. With decreasing amounts of veratrole, the straight portion of the curve is shorter and occurs at lower concentrations of lactic acid. Associated with this, the toe is less accentuated and the break in the upper part of the curve occurs at lower concentrations. In confirmation of Nordbo (*44*) large amounts of veratrole (0.2 ml. of 20 per cent solution) give little color in this range of lactic acid concentration.

In order to obtain direct proportionality between color and quantity of lactic acid, it is necessary to use small amounts of veratrole when small quantities of lactic acid are present and to use larger amounts of veratrole when the quantity of lactic acid is large. In view of the fact that the amount determined clinically by this method will generally fall between 5 and 30 micrograms (corresponding roughly to 7 and 43 mg. per cent in blood), the use of 0.2 ml. of 0.065 per cent veratrole was originally adopted. If larger amounts of lactic acid are to be determined, the sample should be diluted.

QUANTITY OF ALCOHOL ADDED AS VERATROLE SOLUTION. Increasing amounts of alcohol added as veratrole solution or as alcohol prior to the addition of the veratrole solution diminish the final color.

For this reason the authors changed to the use of the optimum amount of veratrole (0.13 mg. or 0.2 ml. of 0.065 per cent solution) in as small a volume of solution as could be measured quickly and accurately. That quantity was 0.10 ml. of 0.125 per cent solution (Figure 3, *E*).

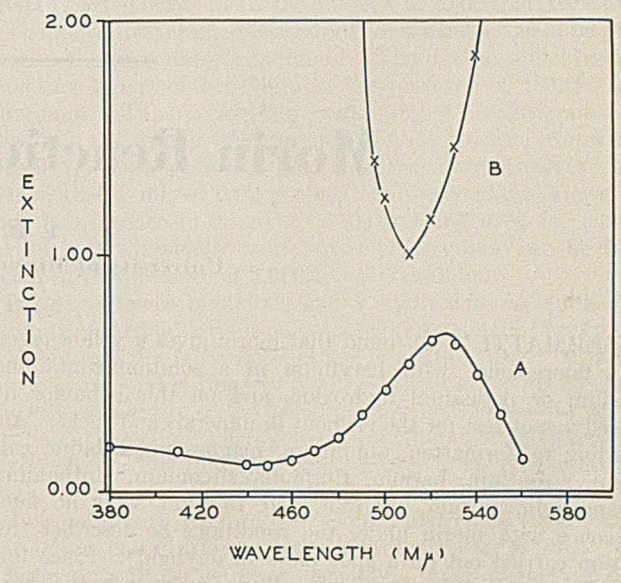

FIGURE 4. SPECTRA

A. Compound formed with veratrole
B. Evelyn 520 filter

TEMPERATURE AND TIME OF COLOR DEVELOPMENT. Nordbo (*44*) has shown that at temperatures between 0° and 5° C. color develops to a maximum in 60 minutes or longer; at temperatures between 10° and 15° C. a maximum is obtained in approximately 20 minutes or more; and at temperatures between 25° and 30° C., a maximum is reached at 20 minutes. The authors have confirmed this work in similar experiments and have found that the maximum color obtained varies inversely as the temperature. The maximum color for the same quantity of lactic acid was approximately twice as strong when developed at 0° C. as when developed at 25° C. The simplicity of maintaining a constant temperature by the use of an ice bath, and the fact that the greatest color is attained at that temperature lead to the adoption of 0° C. for the development of color. At this temperature color increases with time for approximately 60 minutes and then remains constant for at least 1.5 hours. The time finally adopted was 75 minutes.

Color. Numerous spectrophotometric measurements (Coleman and Bausch & Lomb instruments) of the absorption spectrum of the final color were made at different levels of lactic acid content. The spectra obtained with the use of 10 to 90 micrograms were essentially the same and showed a maximum absorption at 5270 Å. (Figure 4). In adapting the method to the Evelyn photoelectric colorimeter, filter 520 and a final volume of approximately 6 ml. were chosen.

Summary

A modification of the Mendel-Goldscheider method for the determination of lactic acid in blood has been developed after a critical review of the methods for lactic acid determinations and a study of the method for blood precipitation, the relation of sulfuric acid and veratrole concentration to the final color, the proportionality of color to concentration of lactic acid, and the adaptation to the Evelyn photoelectric colorimeter.

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Morin Reaction for Beryllium

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ZERMATTEN (*3*) found that morin gives a yellow-green fluorescence with beryllium in a solution containing sodium or potassium hydroxide, and on this behavior he based a spot test for the element in minerals and rocks. According to Zermatten, aluminum, magnesium, lithium, calcium, strontium, barium, titanium, zirconium, lanthanum, dysprosium, cerium, yttrium, and thorium show no fluorescence with morin under the conditions he describes (reaction carried out on a spot plate in daylight). Since few details regarding this test for beryllium are given in the short paper of Zermatten, the reaction has been studied further in this laboratory.

Sensitivity

In 0.01 or 0.1 *N* sodium hydroxide solution the sensitivity of the test corresponds to 0.01 part per million of beryllium when the alkaline test solution having a volume of 10 ml. and containing 0.1 ml. of morin solution (0.02 gram of morin in 100 ml. of acetone) is placed in a 2 × 7 cm. vial and viewed axially in strong daylight (preferably direct sunlight) against a dark shaded background. A very faint fluorescence is then perceptible in the test solution, especially if comparison is made against a blank solution.

The sensitivity of the reaction is greatly increased when the

test solution is examined in ultraviolet light. In the present work a screened mercury glow lamp with a purple Corex glass shell (Central Scientific Company, Chicago) was found to be a satisfactory source of ultraviolet light. When the vial containing the test solution is held in the light of this lamp and viewed transversely, approximately 0.001 part per million of beryllium can be detected when the solution is 0.01 *N* in sodium hydroxide (approximately the optimum concentration). The test solution must be compared with a blank containing the same amount of sodium hydroxide and morin. Morin alone shows a faint fluorescence (yellow-brown) in ultraviolet light and this limits the sensitivity of the beryllium test. Since 1 or 2 ml. of solution suffice for examination, the absolute amount of beryllium detectable is of the order of 0.001 microgram.

With an increase in the sodium hydroxide concentration of the solution, the sensitivity of the reaction decreases. In 1 *N* sodium hydroxide solution the limiting concentration is approximately 0.1 part per million of beryllium in strong daylight, and 0.02 part per million in the ultraviolet light of the glow lamp.

Specificity

In testing the specificity of the morin reaction for beryllium, the solution of the metal ion in question was made 0.5 to 1 *N* in sodium hydroxide (containing sodium carbonate as impurity) and any precipitate formed was filtered off. Morin was added to the filtrate and the solution was examined for fluorescence both in daylight and in ultraviolet light, a blank containing the same amount of sodium hydroxide and morin being used for comparison. The amount of metal present was usually 10 mg. in a final volume of 5 ml. in the case of the common ions; smaller amounts, of the order of 1 mg., were used when the rarer metals, such as indium, were tested.

When tested this way the following elements gave no reaction either in daylight or in the ultraviolet light of the mercury glow lamp: sodium, potassium, rubidium, cesium, magnesium, strontium, barium, cadmium, mercury (Hg^{I} and Hg^{II}), lanthanum, aluminum, gallium, indium, thallium (Tl^{I}), titanium, zirconium, cerium (Ce^{III}), thorium, tin (Sn^{II}), lead, vanadium (V^{V}), arsenic (As^{III} and As^{V}), antimony, bismuth, chromium (Cr^{III}), molybdenum (Mo^{VI}), tungsten (W^{VI}), uranium (U^{VI}), iron (Fe^{III}), cobalt, nickel, palladium (Pd^{II}), and platinum (Pt^{IV}). Columbium and tantalum gave no reaction when the oxides were fused with sodium hydroxide and the filtered leach was tested with morin.

Copper (Cu^{II}), silver, gold, and manganese oxidize morin in sodium hydroxide solution and destroy the reagent. In the case of copper and manganese enough of the metals may remain in solution after filtration of the alkaline medium to destroy the reagent rapidly. Chromate also appears to oxidize the reagent, because beryllium gives no fluorescence in the presence of an appreciable amount of this ion.

The metals that give a fluorescence, similar to that of beryllium, with morin in basic solution are lithium, calcium, zinc, and scandium. The fluorescence of lithium and calcium under the conditions described above is not perceptible in daylight, but is fairly strong in ultraviolet light. The fluorescence given by zinc can be seen in daylight (*1*), but is much less intense than that of a comparable amount of beryllium. Ten milligrams of zinc in 5 ml. of 0.5 *N* sodium hydroxide solution exhibit approximately the same fluorescence in daylight as 1 to 1.5 micrograms of beryllium. The fluorescence of zinc with morin can be destroyed by the addition of cyanide. The solubility of scandium hydroxide in 1 *N* sodium hydroxide is sufficiently great to cause the filtrate to give a very faint fluorescence in daylight. Attempts to make the precipitation of scandium more complete by the prior addition of ferric iron were not entirely successful—for example a mixture of 0.2 mg. of scandium and 3 mg. of ferric iron in 5 ml. of 1 *N* sodium hydroxide gave a filtrate showing a fluorescence equivalent to about 0.2 microgram of beryllium. Better results were obtained by using cobalt in place of ferric iron, possibly because the ionic radii of cobalt and scandium are equal or nearly so. The filtrate from 0.2 mg. of scandium and 1 mg. of cobalt in 5 ml. of 1 *N* sodium hydroxide exhibited about the same fluorescence as a blank, or perhaps slightly stronger than a blank.

The filtrate from yttrium hydroxide shows a very faint fluorescence with morin. Thus 5 ml. of a solution containing 1 mg. of yttrium nitrate (impure) which had been made 1 *N* in sodium hydroxide gave a filtrate which showed a very faint fluorescence equivalent to about 0.2 microgram of beryllium. The fluorescence was visible only in ultraviolet light. It is not certain that the faint fluorescence was actually due to yttrium, but the matter was not further investigated. It has been stated (*2*) that lutecium and ytterbium hydroxides are slightly soluble in sodium hydroxide solution, and these metals may therefore give a fluorescence with morin under the conditions described.

Calcium intensifies the yellow color shown by morin in basic solution, even when present to the extent of 1 part per million.

Beryllium Test in the Presence of Other Elements

No attempt has been made to study the detection of beryllium in the presence of all metals, but the following observations may be recorded. Metals which do not yield insoluble hydroxides or whose hydroxides are soluble in excess sodium hydroxide (with the exception of zinc) do not in general interfere with the beryllium-morin reaction carried out in daylight. There is no difficulty in detecting 0.5 microgram of beryllium in 5 ml. of solution containing, respectively, 10 mg. of antimony (Sb^{III}), arsenic (As^{V}), lead, molybdenum, tungsten, tin (Sn^{II}), vanadium, and lithium if the solution is treated with a few drops of 6 *N* sodium hydroxide, or enough to dissolve any precipitate which is first formed, and the solution is examined for fluorescence in strong daylight against a dark background. The fluorescence given by zinc can be destroyed by cyanide. A solution containing 1 mg. of zinc, 0.05 microgram of beryllium, 1 ml. of 1 *N* sodium hydroxide, and 1 ml. of 5 per cent potassium cyanide in a total volume of 5 ml. shows a rather strong fluorescence in ultraviolet light; a similar solution containing no beryllium and 1 mg. of zinc shows no fluorescence. Accordingly it is easy to detect 1 part of beryllium in the presence of 20,000 parts of zinc.

The morin test is an excellent one for the detection of beryllium in the presence of aluminum. There is no difficulty in detecting 1 part of beryllium in the presence of 100,000 of aluminum. Thus a solution containing 0.1 microgram of beryllium and 10 mg. of aluminum in 5 ml., treated dropwise with 2 *N* sodium hydroxide until the precipitate dissolves and then with 0.1 ml. of 0.02 per cent morin solution, shows a faint fluorescence in daylight, a blank being used for comparison. In ultraviolet light 1 part of beryllium can be detected in the presence of 2,000,000 of aluminum.

In the presence of metals giving hydroxides insoluble in sodium hydroxide, it is usually possible to detect beryllium in the filtrate if a fairly large excess of alkali is used. In this case it is preferable to carry out the test in ultraviolet light, since the sensitivity of the beryllium test is decreased by high alkalinity. One-half microgram of beryllium can be detected in the presence of 10 mg. of ferric iron in a volume of 5 ml. when the precipitation of ferric hydroxide is made at the boiling point with 1 ml. of 6 *N* sodium hydroxide. Approximately one half of the beryllium is present in the filtrate. It is more difficult to separate beryllium from magnesium in this way. A faint test for beryllium can be obtained when 5 ml. of a solution containing 0.5 microgram of beryllium and

10 mg. of magnesium are precipitated hot with 1 ml. of 6 *N* sodium hydroxide, and the filtrate is examined in ultraviolet light after the addition of morin; approximately 80 per cent of the beryllium is coprecipitated with the magnesium hydroxide. The coprecipitation of beryllium is diminished if aluminum is simultaneously present in the solution. In 5 ml. of solution containing 10 mg. each of aluminum and magnesium, it is possible to detect 0.2 microgram of beryllium by precipitating in the way described.

When manganese and copper are present, 1 ml. of stannite (5 per cent stannous chloride dihydrate in 2.5 *N* sodium hydroxide) should be added to the filtrate to prevent oxidation of the morin.

The detection of beryllium in the presence of calcium presents some difficulties. It is possible to detect 5 micrograms of beryllium in the presence of 10 mg. of calcium by adding 4 or 5 ml. of 1 *N* sodium hydroxide (containing the usual amount of sodium carbonate as impurity) to 1 ml. of the solution, filtering off the calcium carbonate, and examining the filtrate in daylight after adding morin. It is not possible to use ultraviolet light for the examination, because the small amount of calcium remaining in solution gives a fluorescence. Alternatively, the precipitation of calcium carbonate can be prevented by the addition of sodium pyrophosphate which, when present in sufficient amount, prevents the fluorescence of calcium in ultraviolet light. Unfortunately, only a comparatively small amount of calcium may be present, for otherwise the originally clear pyrophosphate solution rapidly becomes turbid on standing. A solution containing 2 mg. of calcium, 0.2 microgram of beryllium, 3 or 4 ml. of saturated sodium pyrophosphate, and 0.5 ml. of 1 *N* sodium hydroxide in a total volume of 5 ml. shows a faint fluorescence with morin in strong daylight. A precipitate soon forms in such a solution and the examination must be made without delay. It is difficult to work with a solution containing much more calcium than this. By using ultraviolet light slightly less beryllium can be detected. A solution containing 1 mg. of calcium, 3 or 4 ml. of saturated sodium pyrophosphate solution, and 0.5 ml. of 1 *N* sodium hydroxide in a total volume of 5 ml. shows no more fluorescence in ultraviolet light than a similar blank. One milligram of magnesium in 5 ml. of saturated sodium pyrophosphate solution, together with 0.5 ml. of 1 *N* sodium hydroxide solution, shows a slight fluorescence in daylight equivalent to approximately 0.2 microgram of beryllium.

It is not possible to add tartrate or citrate to solutions containing iron, titanium, etc., with the object of preventing the precipitation of the hydroxides of these metals in basic solution, because magnesium gives a fluorescence with morin in such a medium.

Alkali fluorides, phosphates, silicates, and borates do not interfere with the beryllium reaction.

Summary

The morin-beryllium fluorescence reaction first described by Zermatten has been further investigated. Zinc also gives a fluorescence with morin in daylight in a strongly basic solution, but cyanide destroys this fluorescence without affecting the beryllium fluorescence. In ultraviolet light calcium and lithium show a fluorescence with the reagent under the conditions of the beryllium test; small amounts of calcium can be prevented from interfering by the addition of sodium pyrophosphate.

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Systematic Qualitative Organic Microanalysis

Determination of Specific Gravity

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IN THE identification of an unknown organic liquid, the specific gravity is one of the most important physical constants. Most available methods for the determination of specific gravity are designed for special purposes and do not meet the requirements which have been proposed for the systematic scheme of qualitative organic microanalysis (*2*, *3*, *6*, *9*). An accuracy of from 0.1 to 1 per cent is considered satisfactory for the purpose of identification. To cover a wide range, the upper limit for the amount required is arbitrarily set at 100 cu. mm. and the lower limit at 6 cu. mm. These comparatively large volumes must be recovered after each experiment, in order that the same sample may be available for the determination of additional physical constants, for elementary analyses, or for the preparation of derivatives.

During recent years the author compared many procedures for the determination of specific gravity—e. g., the methods of the falling drop, the submerged float, suspension or flotation (*10*, *22*, *25*), balanced liquids (*14*), and the *schlieren*, and various micropycnometers. Because of the limited range of application of most of these procedures, references to which may be found in the review by Blank and Willard (*12*), only the last two methods have been studied in detail.

The *schlieren* method (*4*, *8*, *18*) allows the determination of the refractive index (*6*) as well as the specific gravity on a few cubic millimeters of the liquid sample: For specific gravity the sensitivity is about 0.0001, and the accuracy is about 0.0005. In colorless liquids the difference between the refractive indices of liquid to be tested (fluid sample) and reference liquid (static sample) makes the direction of flow discernible; in the case of identical specific gravities a cloud effect around the end of the capillary is observed, especially if the tip of the capillary pipet containing the unknown fluid sample is bent at a 90° angle. If the Δn becomes so small as to make the observation of *schlieren* difficult, a trace of a dyestuff added to the fluid sample clearly shows the colored path of flow but does not alter the specific gravity.

High sensitivity, quick performance, inexpensive equipment, and absence of complicated temperature adjustments—the fluid sample acquiring immediately the temperature of the static sample—are characteristics of this procedure. The method, however, is not recommended for general purposes, because the substance usually is lost entirely or recovered with difficulty and numerous reference liquids of known specific gravity must be available; other disturbing factors have been observed when working with aqueous solutions (*4*, *18*). For special cases of

specific gravity determinations the *schlieren* method has been applied by other investigators (*16*, *27*).

The earliest micromethods are based on the use of small pycnometers (*12*) because the pycnometric method appears to be the most precise. Capillaries are widely used as containers for the smallest amounts of liquid samples in individual determinations (*11*, *19*) but have the disadvantage of being fragile. Micropycnometers of the pipet type (*13*, *15*), especially Pregl's precision weighing pipets (*26*), are the most practical apparatus. Subsequent to the author's first description of such pipets (*5*, *24*), Furter (*21*) gave a detailed account of a similar type of micropycnometer pipet especially suitable for measurements at high temperatures.

Specific Gravity Pipets

Two different types of pipets are recommended: One holds a definite volume—i. e., 0.1 ml.—and therefore is used in decigram procedures; the other type serves for the measurement of varying volumes according to the amount of sample available and is recommended in two sizes—i. e., as a centigram pipet for volumes from 20 to 80 cu. mm., and as a milligram pipet for volumes from 6 to 16 cu. mm.

Decigram Pipet. The dimensions of the pipet (Figure 1, *a*) correspond to those of ordinary precision weighing pipets (*26*), but the capillary end has a bore of 1 mm. instead of the usual 0.5 mm. for use with highly viscous oils. The constriction above the bulb is 10 mm. long, is provided with the calibration mark, and has a bore of 0.5 mm. which widens to 3-mm. diameter and finally ends in a tip with 1-mm. bore. Tight-fitting ground caps are placed over each end to prevent loss by evaporation. The weights of these pipets vary from 4 to 6 grams. A somewhat similar pipet with two ground caps, but of larger capacity (0.75 ml.), was used by McLean and Adams (*23*) in the density determinations of substances which melt without decomposition.

Centigram and Milligram Pipets. Since pipets with a bore less than 0.5 mm. are very difficult to fill with viscous compounds, the centigram pipet (Figure 1, *b*) has been designed with a bore of 1 mm. The milligram pipet has the same dimensions except that the bore is 0.5 mm., the lowest practical limit, although Furter (*21*) recommends an inner diameter of only 0.2 mm. The pipets are made of soft glass or "Jena-Geraete Glas", tubing of uniform bore and circular cross section being selected; Jena KPG tubing is recommended for its uniformity. The inner diameter of a piece of glass tubing 150 mm. long may be determined by holding the glass tubing with a capillary clamp (*1*) in the optical axis of the microscope and measuring the diameter with an eyepiece micrometer.

The pipets have an outer diameter of 5 mm. and an over-all length of 120 mm. They are calibrated in 1-mm. intervals. The first mark is placed just above the ground joint, about 20 mm. from the tip; every fifth mark is drawn all around the tube and every tenth mark is numbered consecutively—2, 3, etc., or 20, 30, etc. The satisfactory performance of the two types of pipets depends to a great extent on the correct construction of the ground-joint tips and caps, which are necessary to prevent excessive evaporation of volatile liquids and moisture uptake by hygroscopic substances during weighing.

The ends of the pipet have an outer diameter of about 2 mm. over a length of 6 mm., the outside edges being carefully fire-polished without constricting the inner diameter. The conical part starts 6 mm. from the end of the pipet, widening over a length of 12 mm. to 5 mm. outer diameter. Over a length of from 6 to 8 mm. in its wider part the ground joint fits snugly into the caps. A space of about 1 mm. should remain between the tip and the inner surface of the cap, so that they never touch at any point. Unless there is a minimum of dead space, the liquid column is pressed out at the open end, when the first cap is placed in position. Both caps are numbered to avoid their exchange.

This construction of the caps permits measurements on liquids of low surface tension, such as ether, methyl alcohol, and chloroform, which will flow freely in one direction or the other if the pipet is not held in an exactly horizontal position. Usually it is difficult to prevent some of the liquid from flowing out of a tip during the adjustment of the second ground cap; this amount is collected in the cap but should not come in contact with the rough surface of the ground joint. If the joint becomes wet, even the ground cap will not prevent the evaporation of highly volatile liquids, such as *n*-pentane, trimethyl ethylene, ether, etc.

The pipets are equipped with rubber tubing, a mouthpiece filled with some drying agent, and a counterpoise of approximately the same shape and surface (glass rod): the weights of the pipets vary from 6 to 8 grams. Various pipets were constructed before this final form was found to be satisfactory. (Most of them were supplied by Carl von Czoernig, Radnor, Penna., and Paul Haack, Vienna.)

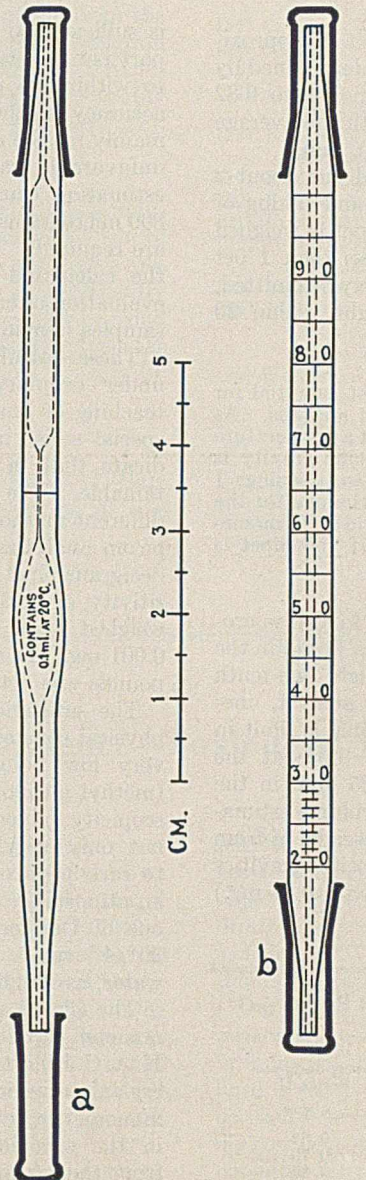

Figure 1. Specific Gravity Pipets (Schematic)

a. Decigram pipet, capacity 100 cu. mm.
b. Centigram pipet if bore = 1 mm., capacity 20 to 80 cu. mm.; milligram pipet if bore = 0.5 mm., capacity 6 to 16 cu. mm.

Calibration of Pipets

Decigram Pipet. The capacity is determined with distilled water in the usual way; heavier liquids, such as bromoform ($d_4^{20} = 2.893$), permit a higher degree of accuracy in the standardization if their specific gravity is known precisely. The use of mercury in such pipets is rather difficult, unless its rapid movement is adequately regulated—e. g., by means of the very useful device of Francis (*20*). In a calibration or determination it is best to bring the meniscus just up to the mark, instead of overshooting and bringing it back.

The constancies of such a decigram pipet are rather astonishing. With regard to weight, a constancy within ±8 micrograms was obtained on 6 consecutive days (readings: tare plus 1.435, 1.435, 1.420, 1.425, 1.421, 1.419 mg.); with regard to capacity, constancy within ±0.1 cu. mm. was obtained by three investigators (0.1019, 0.1021, 0.1019 ml.). After 6 months' use the value changed from 0.1019 to 0.1014 ml., and differences by standardizing with water and bromoform were small—e. g., 0.1012 and 0.1014 ml. found for water, 0.1013 and 0.1015 ml. found for bromoform.

Centigram and Milligram Pipets. In general, the measurement of the bore by means of the microscope is sufficiently

accurate to calculate the volume. It is advisable, however, to calibrate the pipets at the first mark and four other points with distilled water at the standard temperature of 20° C. The water is kept in a test tube in a thermostatically controlled bath; after being weighed on the microchemical balance within ±20 micrograms, the pipet, with the rubber tubing attached to the upper end, is placed in the test tube. About 15 minutes is sufficient time to acquire the temperature of the bath. By means of suction the liquid is drawn to one of the marks, and the pipet is taken out of the test tube and immediately brought into a horizontal position; the free tip is then quickly cleaned from adhering liquid with a piece of chamois. The reading of the meniscus is taken with a low-power lens, at least to about one-quarter division. Finally, the first cap is fitted slowly to the end of the pipet, so that the liquid column is not pressed too far to the other end; after removal of the rubber tubing the second cap is adjusted. The pipet is placed on the hooks of the bow of the left-hand balance pan, and after about 20 minutes is weighed using counterpoise and rider or weights.

The pipet should be handled with capillary forceps or chamois fingers; even then there is a slight heat effect noticeable which apparently diminishes the weight of the pipet if the weighing is carried out immediately after the above manipulations. This procedure is repeated for the other marks and, if necessary, a calibration curve is prepared.

The results obtained with a centigram pipet of approximately uniform bore are shown in Table I. As determined by means of the microscope, one division corresponds to 0.82 cu. mm., a value which compares favorably with the average values of the two series of calibrations—0.83 cu. mm.

The second series of calibrations was carried out about 2 months after the first, with cleaning, drying, and taking of new samples between determinations. The pipet is weighed before each experiment when deviations of less than 1 per cent are desired; otherwise, the first weighing may be omitted, since some of the pipets show constant weight within 20 micrograms over a period of 2 months.

A thermostatically controlled water bath is not essential for preliminary identifications in qualitative organic analysis. As a substitute a 2000-ml. beaker is filled with water at a temperature a few degrees lower than that at which the specific gravity is to be determined. A piece of cardboard with two openings 1 cm. apart serves as a holder for two test tubes, the one for the substance and pipet, the other for 1 ml. of water and a thermometer divided in 0.1° C. The bath is heated and the pipet is filled as soon as the desired temperature is reached.

Discussion of Errors. The major error in the micropipet method is caused when the meniscus of the liquid in the capillary part is read inaccurately. Although one-tenth division can be estimated by the experienced analyst, one-quarter division has been set as an easily attainable limit in these investigations. In the decigram pipet—bore at the calibration mark = 0.5 mm.—an error of 0.25 mm. in the reading causes an error of 0.05 cu. mm. in the volume estimation. This corresponds to a possible error in weight of from 30 to 200 micrograms, when dealing with specific gravities from 0.6 to 4.0. A semimicro balance (sensitivity 0.01 mg.) is sufficient to allow determinations accurate to within 0.5 part per thousand, an ordinary balance (sensitivity 0.1 mg.) to within 1.5 p. p. t. Calculated on the same basis, the accuracy of the centigram pipet with a 1-mm. bore, used mainly for the most viscous liquids, is 10 p. p. t. for the most unfavorable case; since the above error in the volume estimation causes an error in the weight of from 120 to 800 micrograms, only ordinary balances or semimicrobalances are required. For the milligram pipet with a 0.5-mm. bore, the calculated accuracy is within 5 p. p. t. A detailed evaluation of errors with centigrams and milligrams of liquid samples is made by Furter (*21*).

Table I. Calibration of a Centigram Pipet

Number of Divisions from Lower Tip	Temperature Found in Series I	Capacity Calcd. for 20° C., Series I	1 Division Corresponds to: Series I	Series II
	° C.	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*
20[a]	21.0	16.6	0.83	0.82
30	20.0	24.6	0.80	0.79
45	22.0	37.4	0.85	0.85
52	20.5	43.6	0.88	0.87
63	20.0	52.5	0.81	0.81
77	21.0	64.1	0.83	0.83
			Av. 0.83	0.83

[a] First graduation mark 20 mm. from tip.

Table II. Comparison of Specific Gravity Micropipets with Macropycnometer

Substance	Temperatures, ° C.	Specific Gravities: Macropycnometer, 5 ml.	Specific Gravities: Decigram pipet, 0.1 ml.	Specific Gravities: Centigram or milligram pipets[a,b]
n-Pentane	20/4	0.6285	0.628	0.627[b] (12.0 cu. mm.)
n-Heptane	20/4	0.6841	0.685	0.683[a] (45.5 cu. mm.)
n-Butylamine	21/4	0.7400	0.740	0.739[a] (32.0 cu. mm.)
Methyl alcohol	25/4	0.7864	0.785	0.785[a] (28.5 cu. mm.)
Benzene	20/4	0.8790	0.878	0.877[b] (15.0 cu. mm.)
Cyclohexanol	25/4	0.9562	0.954	0.954[a] (25.5 cu. mm.)
Morpholine	20/4	0.9999	1.000	1.005[a] (30.5 cu. mm.)
Aniline	20/4	1.0210	1.021	1.022[b] (9.5 cu. mm.)
Glycerol	21/4	1.2440	1.240	1.245[b] (10.0 cu. mm.)
1,2-Dibromopropane	22/4	1.9300	1.933	1.925[a] (70.5 cu. mm.)
Ethylene bromide	21/4	2.1360	2.133	2.130[b] (13.5 cu. mm.)
Bromoform	20/4	2.8930	2.889	2.890[a] (25.0 cu. mm.)
Clerici solution I	24/4	4.3400	4.330	4.345[b] (8.5 cu. mm.)
Clerici solution II	24/4	4.2230	4.224	4.220[b] (15.0 cu. mm.)

[a] Values determined with centigram pipet, capacity 20 to 80 cu. mm.
[b] Values determined with milligram pipet, capacity 6 to 16 cu. mm.

These calculated accuracies represent the upper limits under ordinary laboratory conditions, especially in the teaching of qualitative organic microanalysis. Results of a special series of experiments, summarized in Table II, indicate that in practice higher accuracies are actually obtainable. The specific gravities were determined by three different methods: (1) standard 5-ml. pycnometer, weighed on an analytical balance with a sensitivity of 0.1 mg.; (2) decigram pipet, weighed on a semimicrobalance with a sensitivity of 0.01 mg.; (3) centigram and milligram pipets, weighed on a microchemical balance with a sensitivity of 0.001 mg. In addition, the specific gravity of over 120 compounds was determined with centigram or milligram pipets.

The substances listed in Table II varied widely in their physical constants: specific gravities between 0.63 and 4.34, very low boiling point (*n*-pentane), low surface tension (methyl alcohol), high viscosity (glycerol), and high hygroscopicity (glycerol, morpholine). For morpholine, which is not only very hygroscopic but also exceedingly sensitive to carbon dioxide from the air, the values were obtained in an atmosphere of nitrogen and compared favorably with the one of Dermer and Dermer (*17*) who reported "0.9994 at 20°/4° vac.". The glycerol contained about 6.5 per cent water, as found by quantitative elementary analysis.

The Clerici solutions mentioned in Table II served as manometer liquids in biological investigations conducted by K. A. C. Elliott (*7*) of this laboratory. During an experiment, crystals appeared in the manometer liquids; errors in the manometric determinations were probably caused by changes in the specific gravity. After removal of the solutions from the manometers the amounts available varied from 8 to 50 cu. mm. Since with larger quantities of substance a higher accuracy of the determination can be expected, the advantages of a pipet with a variable volume range over one with a standard volume become apparent in problems of this kind.

Solid Substances

The specific gravity of solids is important in the field of mineralogy and in investigations of crystalline structure and molecular weight; very little emphasis is placed on its determination in any of the known schemes of qualitative organic analysis. The most suitable procedure for small crystal fragments is the flotation method of Retgers (*25*) which by including centrifugation has been improved by Hendricks and Jefferson (*22*) and by Bernal and Crowfoot (*10*). The latter method has been tested in this laboratory, applying carbon tetrachloride, benzene, nitrobenzene, Clerici solutions, bromoform, and methylene iodide in their pure forms or in mixtures. After centrifuging for 5 minutes, the specific gravities of the reference liquids in which the crystals remained suspended were determined. The amount of material necessary varied over a wide range, between 50 and 250 micrograms, and the accuracy of the determinations was about 5 p. p. t., depending on the accuracy with which the specific gravity of the reference liquid was determined. Since the volumes of the latter after the separation from the solid phase were between 10 and 100 cu. mm., centigram and milligram pipets were applied. Benzoic acid, naphthalene, and resorcinol gave results which agreed favorably with the values reported by Hendricks and Jefferson (*22*).

Conclusion

The general applicability of decigram, centigram, and milligram pipets has been demonstrated for the determination of specific gravities of liquids and solids. The accuracies obtainable under ordinary laboratory conditions are sufficiently high for a safe identification of unknown liquids or solid organic compounds in qualitative organic microanalysis, as shown by their satisfactory use over more than 5 years by different investigators.

Acknowledgment

The author wishes to express his gratitude to W. G. Batt for his help and suggestions during the preparation of this article.

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Presented before the Division of Microchemistry at the 96th Meeting of the American Chemical Society, Milwaukee, Wis.

Correspondence—Toxicity of Aromatics

Sir: Apparently the degree of toxicity of the simpler members of the aromatic series is a subject more involved than we recognized when preparing the article entitled "Detection of Aromatics in Air" [Ind. Eng. Chem., Anal. Ed., **12**, 433 (1940)]. In this article we made the unfortunate error of substituting the word "aromatic" for "benzene" in quoting the National Safety Council Benzol Report of 1926. R. C. Stratton, supervising chemical engineer of the Travelers' Insurance Company, has very kindly pointed out that this error has caused the reproduction of an inaccurate statement: "that 100 p. p. m. of aromatics is the maximum aromatic concentration in which a man may work safely."

The original of this quotation, we now understand, meant that concentrations of benzene in excess of 100 p. p. m. will cause chronic, but not acute, pathological poisoning. Although a number of references in Ethel Browning's "Toxicity of Industrial Solvents", issued by His Majesty's Stationery Office, London, 1937, state that toluene and xylene are at least as toxic as, if not more than, benzene, Mr. Stratton has explained that these statements refer to acute poisoning, and most probably indicate that these heavier aromatics cause more rapid narcosis, and other symptoms of acute asphyxiation or poisoning, than does benzene. Mr. Stratton points out a further reference, which we had overlooked, as follows: "With concentrations of 620 to 1000 p. p. m., inhalation of toluene produces practically no symptoms in animals."

Our article was concerned primarily with a procedure for determining quantitatively the concentration of aromatic vapors in industrial atmospheres and we believe that the method outlined is accurate, reproducible, and valid for this purpose. The decision as to the maximum concentration for a satisfactory working condition does not rest with the chemist, and is apparently a potential subject for wider investigation and education. We are offering this communication to correct any inaccurate impressions which may have been made by the original article.

G. R. Gilbert and R. E. Tannich

Humble Oil & Refining Co.
Baytown, Texas
October 21, 1940

Determination of Mercury in Urine

A Photometric Method Using a New Reagent, Di-Beta-Naphthylthiocarbazone

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THE behavior of di-β-naphthylthiocarbazone toward the salts of heavy metals has been studied recently by Suprunovich, who reported that it was closely analogous to that of dithizone (diphenylthiocarbazone), but characterized by a greater sensitivity of reaction (*10*). Since the mercury complex of di-β-naphthylthiocarbazone is red, a color especially attractive for "mixed color" technique, it appeared desirable to study the properties of this complex with the object of applying this reagent to the determination of mercury in biological material, especially urine.

Both Preund (*9*) and Suprunovich (*10*) have synthesized the compound, the latter using a method similar to that employed by Fischer (*3*) in the synthesis of dithizone. In the author's hands the method of Suprunovich (*10*) was not satisfactory. By following his technique the 2-naphthylhydrazine salt of 2-naphthylhydrazino-β-dithiocarbonic acid (melting point 135° C.) was obtained as reported. However, removal of hydrogen sulfide from the molecule by melting in an atmosphere of carbon dioxide failed to yield di-β-naphthylthiocarbazide with any degree of purity. But when a saturated benzene solution of the 2-naphthylhydrazine salt of 2-naphthylhydrazino-β-dithiocarbonic acid was allowed to stand for several days in a loosely stoppered Erlenmeyer flask, a slow evolution of hydrogen sulfide resulted and di-β-naphthylthiocarbazide was obtained as a gray precipitate adhering to the sides and bottom of the flask. This intermediate was then converted to di-β-naphthylthiocarbazone by following the method as outlined from that point. Microanalytical results for the final product as listed in Table I were computed on an ash-free basis (ash content 1.5 per cent).

TABLE I. ANALYSIS OF DI-β-NAPHTHYLTHIOCARBAZONE

	Found	Theoretically Present
	%	%
C	70.60	70.78
H	4.64	4.53
N	14.98	15.72
S	9.86	8.99

Because of the method of synthesis employed, the ash consisted principally of potassium salts which did not introduce any difficulty into the analytical procedure. Several lots synthesized by this method, as well as a lot synthesized by an entirely different procedure, yielded products which in chloroform solution gave identical transmission curves. For this reason and because the author's analytical results were in close agreement with those of Suprunovich (he found 70.52 per cent for carbon and 4.71 per cent for hydrogen), the author assumed that he was dealing with the compound which Suprunovich had described (*10*). Suprunovich evidently based his identification of the compound on these two elements.

Di-β-naphthylthiocarbazone synthesized as described above became the basis of a photometric "mixed color" method for the quantitative determination of mercury in urine, with a sensitivity comparable to that of similar methods for lead and bismuth employing dithizone (*6*, *7*). Two extraction steps were found necessary, the first to remove copper interference and place the range, the second for the final estimation of mercury as its pure complex with di-β-naphthylthiocarbazone.

Reagents

High-grade chemicals are used throughout the analysis to ensure a low initial mercury content, but no attempt is made to remove traces found in the potassium permanganate.

Chloroform is freshly distilled, and used chloroform containing di-β-naphthylthiocarbazone and the di-β-naphthylthiocarbazone complex of mercury is reclaimed as previously described (*6*), except that 5 ml. of absolute alcohol are added to each liter of reclaimed chloroform to replace alcohol removed during treatment with hydroxylamine.

Glassware

All glassware (Pyrex) before use is washed thoroughly with hot dilute nitric acid (50 ml. of nitric acid, sp. gr. 1.40, per 100 ml.) and rinsed with distilled water to ensure removal of mercury present as surface contamination through previous use.

Apparatus

A photoelectric spectrophotometer, constructed in this laboratory and shown essentially in Figure 1, is employed for transmission and density measurements. Readings are taken with the monochromator set at 515 mμ. The instrument is equipped with Aminco, style D, class 3, high precision matched cells. The light source consists of a 100-watt, 115-volt projection lamp (with prefocused base) maintained at a constant voltage (115 volts) by a Raytheon voltage regulator. Two optically centered double convex lenses are placed in the light path, a 220-mm. focus lens between the light source and the absorption cells, and a 100-mm. focus lens in front of the spectrometer. An automatic shutter is placed in the light path between the cells and the 100-mm. lens. The photocell, a G. E. blocking layer type, placed at the exit pupil of the spectrometer is used with a Type R Leeds & Northrup galvanometer, sensitivity 0.0005 microampere per mm. at a distance of 1 meter from scale.

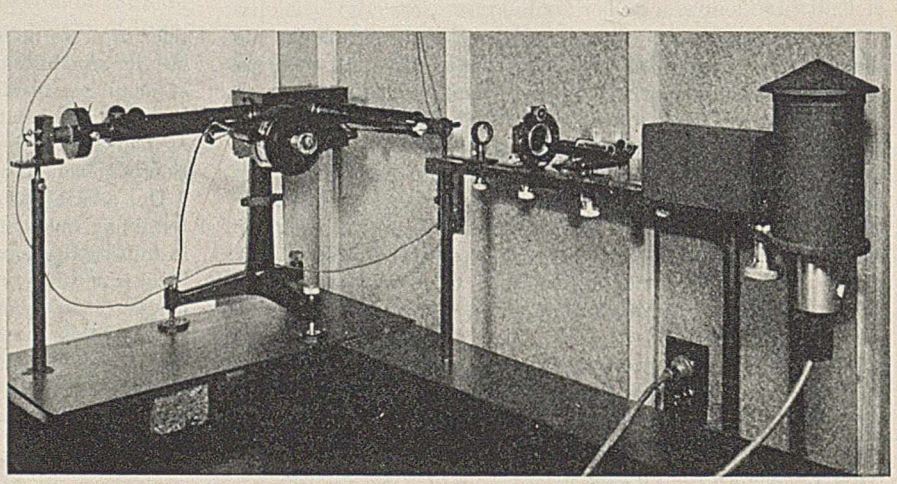

FIGURE 1. PHOTOELECTRIC SPECTROPHOTOMETER

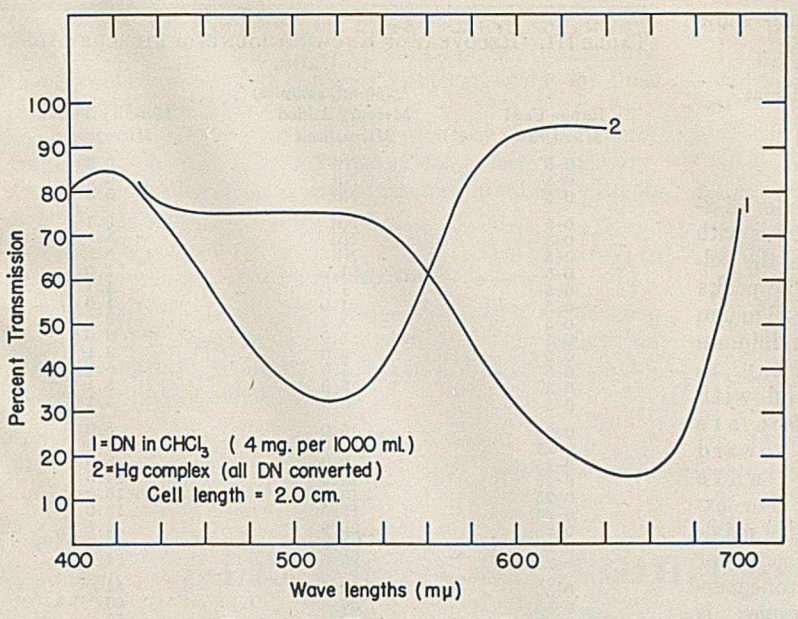

FIGURE 2. TRANSMISSION CURVES

Procedure

PREPARATION OF SAMPLE. Measure 50 ml. of urine and transfer to a 200-ml. boiling flask with round bottom and vial mouth. Add 10 ml. of dilute sulfuric acid (50 ml. of sulfuric acid, sp. gr. 1.84, per 100 ml.) and one 5-grain (0.32-gram) tablet of potassium permanganate. Drop into the flask a few small pieces of Carborundum, insert an all-glass "cold finger" condenser (*2*, *5*) with lower end extending into flask to a point about 1.25 cm. (0.5 inch) above surface of the liquid, and apply heat, boiling gently. When solution clears remove the condenser and quickly add another tablet. Repeat this operation until the potassium permanganate discoloration persists; four tablets generally suffice. Remove the condenser, cool the solution to room temperature, decolorize by the dropwise addition of aqueous hydroxylamine hydrochloride solution (50 grams per 100 ml.), and follow with 2 ml. more. Reinsert the condenser and heat the flask and contents just to boiling. Cool as before and transfer the contents with rinsing to a properly graduated 150-ml. Squibb separatory funnel equipped with glass stopper held on by a rubber tie.

EXTRACTION 1 (Removal of Copper). Dilute the sample, thus prepared, to 100 ml. and extract the mercury together with any small amounts of copper present by the addition of di-β-naphthylthiocarbazone solution in chloroform (20 mg. per liter). First add 2 ml. and shake the funnel and contents vigorously for 1 minute. Visual inspection of the color of the separated chloroform phase will indicate whether or not the mercury content is below 5 micrograms. Continue extraction by adding a further 3-ml. portion of di-β-naphthylthiocarbazone solution, shaking for 1 minute and repeating with 5-ml. portions until color changes are no longer visible, thus fixing the range in a manner similar to that employed for lead and bismuth (*1*, *6*, *7*). The mercury complex of di-β-naphthylthiocarbazone in chloroform imparts a red color with a blue tinge. Each 5-ml. portion extracts approximately 25 micrograms of mercury.

TABLE II. DI-β-NAPHTHYLTHIOCARBAZONE CONCENTRATIONS AND CELL LENGTHS

(For different ranges in the determination of mercury in urine)

Range *Micrograms*	DN Concentration *Mg./l.*	Volume Used *Ml.*	Cell Length *Mm.*
0–5	6	10	50
0–25	8	25	25
0–50	20	20	10

To the combined extracts or an aliquot corresponding to not more than 50 micrograms of mercury contained in a separatory funnel, add a mixture consisting of 75 ml. of water, 2 ml. of dilute sulfuric acid (50 ml. of sulfuric acid, sp. gr. 1.84, per 100 ml.) and 4 ml. of aqueous sodium thiosulfate solution (1.5 grams per 100 ml., *11*). Shake the funnel and contents for 1 minute, thereby transferring the mercury from the chloroform to the aqueous phase (the copper remaining in the chloroform phase), and then discard the chloroform layer. Remove traces of di-β-naphthylthiocarbazone by washing with two or three 2-ml. portions of chloroform and then remove the chloroform completely. Transfer the aqueous fraction completely to the original 200-ml. boiling flask, add 5 ml. of saturated potassium permanganate solution, insert the condenser, and apply heat as before for about 10 minutes. Cool the solution, and decolorize by adding aqueous hydroxylamine hydrochloride solution (5 grams per 100 ml.) drop by drop. Add 1 ml. in excess, reinsert condenser, and heat just to boiling, then cool and dilute to 100 ml.

EXTRACTION 2 (Estimation of Mercury). For the final estimation, extract the mercury by means of chloroform solutions of di-β-naphthylthiocarbazone of various strengths, depending upon the range. The technique of estimation has been previously described (*6*). The concentrations of di-β-naphthylthiocarbazone and the cell lengths used for different ranges may be obtained from Table II.

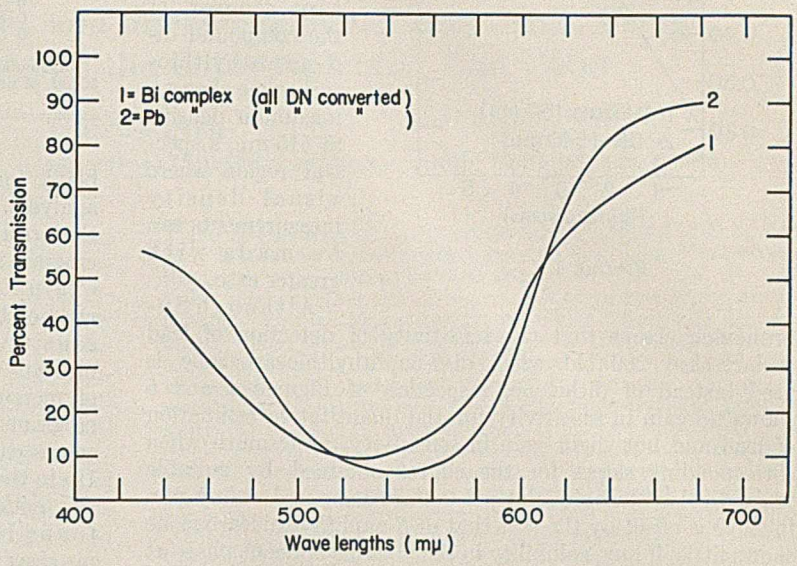

FIGURE 3. TRANSMISSION CURVES

Working curves are obtained with known amounts of mercury as the nitrate. The mercury standards are treated with 1 ml. of aqueous hydroxylamine hydrochloride solution (5 grams per 100 ml.), 2 ml. of dilute sulfuric acid (50 ml. of sulfuric acid, sp. gr. 1.84 per 100 ml.), and sufficient distilled water to give a volume of 100 ml., before proceeding with the extractions.

Analytical Results

In Table III are listed results obtained by the analysis of 50-ml. samples of normal urine (in triplicate) containing known added amounts of mercury. The results reported were obtained by subtracting the reagent blank of 0.5 microgram. The first three results shown in Table III are listed as the actual reagent blank obtained by substituting 50 ml. of distilled water for 50 ml. of urine.

Discussion

The compound di-β-naphthylthiocarbazone exhibits the same general characteristics as dithizone. The chloroform-

soluble complexes with mercury, lead, and bismuth show specific differences in color, however, as shown below:

Metal	Color with DN	Color with Dithizone
Hg	Red (blue shade)	Yellow
Pb	Purple	Rose red
Bi	Magenta	Orange

In Figures 2 and 3 are shown transmission curves for di-β-naphthylthiocarbazone and its corresponding complexes with mercury, lead, and bismuth, chloroform serving as the solvent. The peaks of maximum density (minimum transmission) as compared with dithizone are shifted toward higher wave lengths. For example, the maximum density for the dithizone mercury complex is found at 490 mμ, whereas the mercury complex of the compound di-β-naphthylthiocarbazone shows a maximum density at 515 mμ, a spectral region where visual density measurements can be made with greater ease.

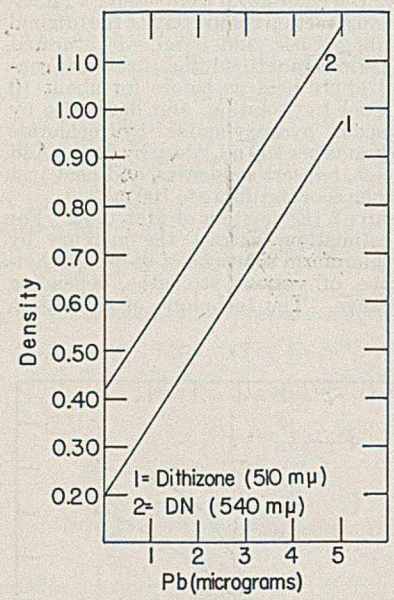

FIGURE 4

Although Suprunovich claims that the sensitivity of detection of lead is increased 200-fold when di-β-naphthylthiocarbazone is used instead of dithizone, inspection of Figures 4 and 5 shows no gain in sensitivity for the quantitative estimation of lead and but slight gain in sensitivity for bismuth when corresponding slopes for the curves obtained by suitable "mixed color" technique at pH 9.5 are compared. Any gain in slope is offset by the fact that di-β-naphthylthiocarbazone shows little, if any, solubility in the alkaline aqueous phase at pH 9.5, as demonstrated by an unusually high density reading for zero lead and zero bismuth.

Fischer (*4*) states that a "mixed color" dithizone method for mercury would be disturbed by the presence of copper, silver, gold, palladium, and platinum. These metals, with the exception of palladium, were tested with di-β-naphthylthiocarbazone and the same interference was found. Gold, palladium, and platinum have not been encountered in urine. Copper can be separated from mercury by means of the thiosulfate ion. The presence of silver in urine has been shown to be very infrequent (*8*) and interference by it has not been encountered in this study.

The method of preparing urine samples by using potassium permanganate and sulfuric acid for the destruction of organic matter is satisfactory. Solid tissues and blood have not been investigated as yet and it is recognized that the destruction of organic matter in these materials requires a more drastic treatment for which special technique must be developed to prevent losses of mercury.

The reagent blank of 0.5 microgram of mercury has been found constant for each analytical result shown in Table III. This constant depends primarily upon the amount of potassium permanganate used, and it is necessary to run a reagent blank for each set of analyses. Because of the error of ±0.1 microgram inherent in the evaluation of this reagent blank, the sensitivity of the method for amounts of mercury of 5 micrograms and below becomes ±0.2 microgram. Up to the present time no method has been found for removing mercury from the potassium permanganate used.

TABLE III. RECOVERY OF KNOWN AMOUNTS OF MERCURY ADDED TO URINE

(50-ml. samples)

Range Used *Micrograms*	Mercury Added *Micrograms*	Mercury Found *Micrograms*
0–5	Nil	0.6[a]
0–5	Nil	0.5[a]
0–5	Nil	0.5[a]
0–5	Nil	0.1
0–5	Nil	Nil
0–5	Nil	Nil
0–5	1.0	1.0
0–5	1.0	1.1
0–5	1.0	1.0
0–5	3.0	3.0
0–5	3.0	3.0
0–5	3.0	3.1
0–5	5.0	5.1
0–5	5.0	5.1
0–5	5.0	5.1
0–25	5.0	5.0
0–25	5.0	4.5
0–25	5.0	5.0
0–25	15.0	14.5
0–25	15.0	15.0
0–25	15.0	15.0
0–25	25.0	24.5
0–25	25.0	25.0
0–25	25.0	24.5
0–50	50	51
0–50	50	50
0–50	50	51
0–50	100	100
0–50	100	102
0–50	100	100
0–50	250	245
0–50	250	250
0–50	250	250

[a] Calculated as a reagent blank, substituting 50 ml. of distilled water for 50 ml. of urine.

A photoelectric spectrophotometer has been employed in the author's experiments; however, visual instruments such as spectrophotometers or photometers with suitable filters may be used for photometric measurement.

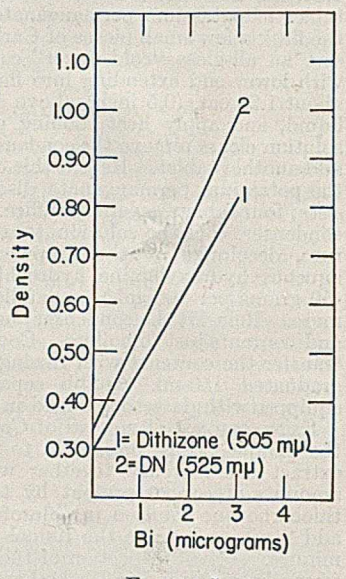

FIGURE 5

Since the yield of di-β-naphthylthiocarbazone by the author's adaptation of Suprunovich's method is not very satisfactory, further work on the synthesis of the compound is going forward.

Summary

A photometric "mixed color" method has been developed for the determination of mercury in urine by the use of di-β-naphthylthiocarbazone, an analog of dithizone.

Small samples (50 ml.) are prepared for analysis by oxidizing the organic matter with potassium permanganate in the presence of sulfuric acid. The mercury is extracted in two

steps, the first to remove copper (the only interfering element found present in urine) and the second to separate the mercury as a pure complex of di-β-naphthylthiocarbazone for final photometric estimation.

The method is very sensitive. An accuracy of ±0.2 microgram has been obtained for 5 micrograms or less of mercury. Amounts exceeding 50 micrograms can be determined with an error not greater than ±2 per cent.

Acknowledgment

The writer wishes to acknowledge the helpful suggestions given by E. W. Scott in synthesizing di-β-naphthylthiocarbazone; also the aid given by J. Cholak in designing the photoelectric spectrophotometer used.

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Titration of Ammonia in Presence of Boric Acid

In the Macro-, Semimicro-, and Micro-Kjeldahl Procedures, Using Methyl Red Indicator and the Color-Matching End Point

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THIS paper supplements a previous communication (*6*), in which there were shown the accuracy of the color-matching end point with methyl red indicator in the titration of ammonia in boric acid solution, and its applicability to the macro- and micro-Kjeldahl procedures. The same titration procedure was later incorporated into the semimicro-Kjeldahl method; the conditions for this titration, not previously published, are given below, with some analytical results. It appears, from inquiries received and from observations of student experiences, that the earlier description of the titration may have been insufficiently explicit, leaving the operator not fully prepared for the color phenomena observed as the end point is approached and reached. The comments and additional directions given below are intended to clarify this matter. The essential experimental conditions for the macro-, semimicro-, and microprocedures are tabulated, so as to have in one place the information needed for execution of the decomposition, distillation, and titration on any of these three scales.

Boric Acid Solution

A 4 per cent solution of boric acid serves for the macro-, semimicro-, and microprocedures. As a matter of convenience, and to ensure the presence of the same quantity of methyl red in both color standard and analysis liquid, the methyl red should be added to the whole supply of boric acid solution, prepared as follows:

Dissolve 40 grams of boric acid in each liter of water, boiling the solution for some time to expel carbon dioxide. Transfer the solution to a bottle or flask of Pyrex glass (*4*) and allow to cool. To determine the proper amount of indicator, add for each liter of solution 2 cc. of a 0.05 per cent solution of methyl red in dilute alcohol (prepared by dissolving the methyl red in 95 per cent alcohol and then diluting with about two thirds as much water). Measure with a graduated cylinder the volume of the boric acid solution required for analysis (Table II, B 2), transfer to an Erlenmeyer flask (Table II, B 1), dilute with water as indicated in Table II (the sum of the quantities given in B 3 and B 4; ordinary laboratory distilled water may be used here), and observe the color, which should be clear red.

To judge whether or not the color is of an intensity favorable for the color-matching titration, introduce 0.5 drop of standard alkali, whose normality is approximately that of the acid to be used (Table II, C 2), and then one drop of the standard acid (or vice versa), and observe whether or not the color changes are readily detectable, making the observations by light from the source to be used in the analyses. Then, as needed, add to the stock solution either more methyl red or more 4 per cent boric acid solution, and repeat the test. The amount of indicator to be used is to some extent a matter of individual preference, but a color which is too strong or too faint is hard to match.

Store the solution so as to avoid undue access of air. It usually keeps well (*4*), and in presence of the indicator any deterioration (*6*) is revealed by a change of color to red-orange or orange—i. e., a color other than clear red. In this case add to the entire supply enough 0.1 *N* acid (by drops) to restore a normal color.

Color-Matching Titration

The titration of ammonia in boric acid solution, with the aid of methyl red indicator, is based on the facts that in presence of boric acid the indicator develops its acid color in an intermediate intensity, corresponding to the acidity of the boric acid solution, and that this color is markedly changed in intensity by minimal amounts of either alkali or mineral acid. During the titration of ammonia the initially yellow or orange solution develops a reddish cast as the equivalence point is approached, and upon continued addition of acid the red tone becomes progressively clearer and deeper, finally reaching a maximum well beyond the equivalence point. There is at no time a sharp color change, but at the equivalence point the intensity of the red color is identical with that of the boric acid–methyl red solution similarly diluted. This point of equal intensities can be recognized by having at hand as a guide for the eye a properly prepared color standard which contains in the same volume of liquid the same amounts of boric acid and methyl red. Most persons can readily detect

TABLE I. DETERMINATION OF NITROGEN BY SEMIMICRO-KJELDAHL METHOD[a]

(With absorption and titration of ammonia in boric acid solution)

Substance	Nitrogen Calculated	Nitrogen Found
	%	%
Taurine	11.20	11.19 11.11
Trimeric methylene-*p*-phenetidine	9.39	9.23 9.31
Methylene-*N*,*N'*-bis-(*p*-phenetidine)	9.78	9.73 9.72
3-*p*-Anisyl-6-methoxy-1,2,3,4-tetrahydroquinazoline	10.37	10.34 10.30 10.26
N-[(2-benzalamino-5-chloro)-benzyl]-*p*-chloroaniline	7.89	7.80 7.84
$(C_6H_5CH_2.N.C_6H_4CH_2-)_3$	7.17	7.11 7.07

[a] Analyses represented were performed by F. W. Landau.

the differences in color intensity corresponding to 0.01 to 0.02 cc. of standard acid.

To increase the accuracy of observation the Erlenmeyer flasks used should be identical in capacity and shape, and should be as nearly as possible alike in wall thickness and in the character of the shadows or optical aberrations observable within their liquid contents.

In the macro- and semimicroprocedures the boric acid–methyl red solution may be measured with a graduated cylinder; a pipet should be used in the microprocedure. To minimize final error due to carbon dioxide the water added in preparing the color standard should be (*a*) carbon dioxide–free water approximately equal in volume to the water transferred during the Kjeldahl distillation, and (*b*) ordinary distilled water in approximately the amount of the titration. Since an approximate equalization of volumes is frequently necessary near the end point, it is advisable to prepare the color standard so that its volume is slightly greater than the anticipated final volume of the titration liquid. The equalization of volumes then involves only the addition of water to the titration liquid, and the color standard need not be altered. The properly diluted color standard (Table II, B), if kept in a tightly stoppered flask, can be used for a week or more or until a noticeable alteration of the original color occurs.

At the end of the Kjeldahl distillation place the flasks containing the ammoniacal distillate and the color standard side by side on the white surface of the buret stand. The two liquids must be equally lighted, and the titration should be made in a place where no near-by objects cause unequal shadows within the flasks. Daylight or light from a titration illuminator is to be preferred, but titrations have been made by ordinary electric light with not more than slight decrease in accuracy. Titrate the ammonia with standard acid until a pink tint appears and deepens to an intensity still plainly weaker than that of the control.

Now approximately equalize the volumes of the two liquids (equalization of liquid levels will suffice) by addition of water to the partially titrated distillate, using water free from carbon dioxide if much is required. Continue the titration cautiously thereafter, by small increments and finally by drops and fractions of drops, until the red color matches in intensity that of the color standard. The intensities can be matched readily by looking obliquely downward through the liquids at the white surface beneath. It is best to view the two liquids simultaneously and to make an immediate decision as to the equality or inequality of the two intensities. When the colors appear to be identical record the buret reading, and then test the end point by addition of 0.01 to 0.02 cc. of standard acid, which should markedly and unmistakably increase the color intensity as compared with that of the control. It is an advantage of this procedure that the end point can be confirmed within 0.01 to 0.02 cc. by the overtitration described.

TABLE II. ESSENTIAL EXPERIMENTAL CONDITIONS FOR MACRO-, SEMIMICRO-, AND MICRO-KJELDAHL PROCEDURES

(With absorption and titration of ammonia in boric acid solution)

		Macro	Semimicro	Micro
A.	Digestion and distillation of ammonia			
	1. Kjeldahl flasks, capacity, cc.	500	100	15
	2. Nitrogen, normal limits, mg.	15 to 90	1.5 to 7	0.4 to 1.4
	3. H_2SO_4 concd., most compounds, cc.	20–30	3–5	1
	4. K_2SO_4, grams (or 0.8 as much Na_2SO_4)	10	1–2	0.4[c]
	5. Catalyst[a]	Se 0.2 g. HgO 0.5 g.	Se 0.05 g.[b] HgO 0.1 g.	Se 0.02 g.[c] HgO 0.03 g.
	6. Normal time of digestion after clearing, min.[d]	60	25	15
	7. Water added, cc.	150–200	35–50	6[e]
	8. Volume distilled, cc.	150	25	10[f]
B.	Color standard			
	1. Erlenmeyer flasks, capacity, cc.	500	100 or 150	50 or 100
	2. Boric acid–methyl red solution, cc.	50	25	5
	3. CO_2-free water, cc.	150	25	10
	4. Ordinary distilled water, cc.	50	25	10
C.	Titration of ammonia			
	1. Buret, capacity and graduations, cc.	50/0.1	25/0.1	10/0.05
	2. Normality of acid	0.1–0.2	0.02	0.01
	3. Limits of titration, cc.	10 to 50	5 to 25	3 to 10
	4. Volume at end point, cc.	210 to 250	55 to 75	18 to 25

[a] For most analyses selenium alone is effective. For substances difficult to decompose, both selenium and mercury may be used to advantage (*2*, *7*, *9*). Rapid digestions are claimed by use of mercury with dipotassium phosphate and ferric sulfate (*11*). To precipitate mercury there should be added, at the time the liquid is made alkaline, for each 0.1 gram of mercuric oxide not less than 0.12 gram of crystalline sodium sulfide, 0.8 gram of fused chips, or 2.2 grams of sodium thiosulfate (*3*).

[b] It is convenient to use a selenized boiling granule, as supplied by the Hengar Company, 1833 Chestnut St., Philadelphia.

[c] The potassium sulfate–copper sulfate mixture recommended by Pregl (*8*, *10*) may be used, the decomposition being hastened by addition of perhydrol. Instead, there may be used about 0.4 gram of a mixture made by grinding together 25 grams of potassium sulfate and 1 gram of selenium; if desired 2 grams of mercuric oxide may be included.

[d] The proper duration of the "afterboil" is disputed. In general it is probably unsafe to heat only to clearing of the acid mixture, as has several times been recommended. For certain more refractory nitrogen compounds—e. g., derivatives of quinoline, carbazole, quinazoline, quinoxaline, and substances such as sultams, casein, etc.—the afterboil suggested in the table must be considerably extended.

[e] Includes water used to wash the diluted acid into distilling vessel.

[f] It is probably better practice to collect a definite volume of distillate than to distill for a definite time (*1*, *5*).

Semimicroprocedure

The semimicro-Kjeldahl method as used in this laboratory is conducted in all important respects like the macroprocedure, with suitable decreases in size of apparatus, quantities of reagents, etc. The results obtained during a number of years appear to be quite as satisfactory as those obtainable by the macroprocedure. Details of the semimicroprocedure may be omitted, but essential quantities, etc., are given in Table II, and some test results are listed in Table I.

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Microapparatus for Fractional Recrystallization

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FOR the purification and separation of small amounts of material by fractional crystallization the chemist too frequently searches for a solvent in which the materials are slightly soluble, in order that a comparatively large volume of solvent may be used in familiar apparatus. A number of objections are at once apparent in such a procedure. In the first place, extraneous solid material such as dust particles, lint, etc., which are always difficult to keep out, will be proportionately present in many times the amount and will be collected along with the small amount of precipitate. Impurities introduced with the solvent itself will be also proportionately larger and special precautions as to the purity of the solvent must be taken. Finally, the recrystallization may correspond more nearly to a precipitation in which the desired purification is not achieved. Recrystallization of small quantities of material from concentrated solution requires the manipulation of correspondingly small volumes of liquid. This is easily accomplished when suitable centrifuge filtration technique is used.

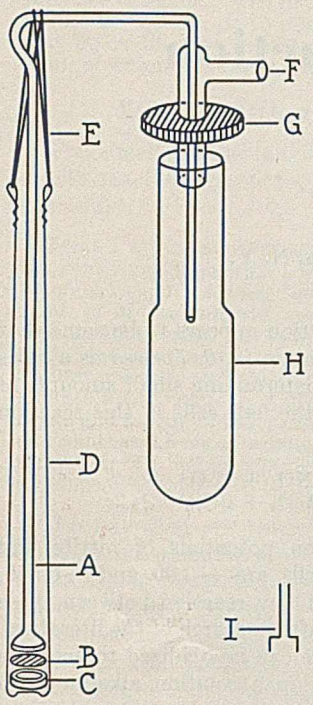

FIGURE 1

A customary practice is to add varying amounts of a second solvent to reduce the solubility and thus cause crystallization. Once crystallization has set in, the proportions of the solvents are not known unless measured volumes have been used and no concentration is resorted to. However, it is frequently desirable to reduce the volume by evaporation or distillation. In such case, the correct wash solution is not exactly known, and the mixture employed often either dissolves more of the crystalline material than is necessary or precipitates sticky material on the crystals. The centrifuge filtration apparatus is free from this objection, since practically no mother liquor adheres to the crystals and washing is not necessary.

Most of the disadvantages of filtration by suction have been pointed out by Skau (*3*), who devised a suitable centrifuge filtration apparatus and enumerated the advantages of such a technique. The advantages are much more apparent with small volumes, until a point is reached when clear-cut filtration by suction can no longer be accomplished. In order to carry on from this point the technique of recrystallization from melting point tubing was devised by Emich and Hausler (*1*).

This paper describes a few simple pieces of apparatus suitable for use in this connection which will not necessarily require the services of an expert glass blower to construct.

Nearly always at the outset of purification by recrystallization and always just before the final recrystallization previous to analysis it is desirable to filter a solution of the material in order to remove lint and other solid impurities. For this purpose some form of the filter stick is most useful. The one shown in Figure 1 is easily constructed.

A is a straight glass tube approximately 2 mm. in diameter and long enough to reach to the bottom of any test tube. Its lower end is broadened out to a diameter of 3 or 4 mm. and ground absolutely flat, so that the small filter paper, *B*, will be properly seated on it. *C* is a glass ring of the same diameter as the filter paper and a depth of approximately 1 mm. The surface of *C* which is next to the filter paper is also ground flat in order to hold the filter paper evenly against the opposite ground surface. *C* is pressed firmly in place by a wire loop, *D*, which is in turn held in place by the rubber band, *E*. *D* is made from either platinum or Nichrome wire.

The upper end of *A* is bent in the shape represented, so that it leads directly into the crystallizing vessel, *H*. The mouth of *H* is not necessarily ground, but is smooth and even, so that when suction is applied at *F* it will fit tightly against the flat undersurface of the rubber collar, *G*. Thus, filtration is effected directly into the crystallizing vessel, which is a part of the centrifuge filtration apparatus illustrated in Figure 2 (left) for the collection of the solid crystalline material. The necessary amount of concentration can usually be carried out in the crystallizing vessel.

An alternative way of constructing the end of the filter stick which holds the filter paper is shown by *I*. Here a flat shoulder to provide a resting place for the filter paper is made by enlarging the glass tubing with a carbon rod cut off squarely at one end. The enlarged portion extends downward beyond the shoulder a distance of approximately 2 mm. After the filter paper is inserted a wad of cotton is pressed snugly in below it in order to hold the paper in place.

An assembly for the collection of crystals is shown in Figure 2 (left). *H* is the crystallizing vessel. A few centimeters from its mouth a shallow shoulder is constructed for its support during centrifugation. This shoulder rests directly on a collar, *K*, made from soft lead or tin, which in turn rests on the top rim of the filter, *L*. The lower end of *L* is funnel-shaped. The narrow lower part of *L* is closed by a glass plug, *M*, of shape represented in the drawing. The lower end of the plug is enlarged by holding its tip in the flame after it is inserted in the funnel, so that it cannot fall back through when the apparatus is inverted. The plug, *M*, is not ground in and thus leaves sufficient space around it through which only unusually fine crystals will pass. Ordinarily, during centrifuging a few fine crystals will pass through before the larger crystals themselves form a filter mat, but this is unimportant, since nearly always some of this material is dissolved in the mother liquor. For fine crystals, filter *L* can be replaced by a sintered filter of the proper size (*2*). *L* rests on a collar of soft lead or tin, *N*, which in turn is supported by the shoulder, *O*. *O* is easily formed by heating the test tube which forms the outside of the assembly, in a narrow flame at the point indicated.

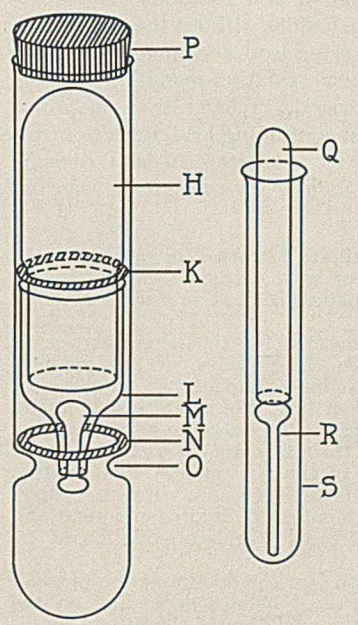

FIGURE 2

For assembly just before use the apparatus is in the reverse position to that shown in the diagram, so that the crystallizing vessel will be in an upright position and rest on the rubber stopper, *P*, which closes the assembly. The entire apparatus can then be

immersed in a cooling liquid at any desired temperature for crystallization. Following crystallization the assembly is inverted and quickly centrifuged.

When smaller amounts of material are to be handled, the simpler apparatus shown on the right of Figure 2 has been found more desirable.

The crystallizing vessel, Q, is a small-sized test tube 6 mm. or less in diameter, with a perfectly symmetrical opening. R, the filter rod, is an ordinary glass rod heated in the flame until its upper end forms a symmetrical round ball of such size that the test tube will not be able to slip over it. These two parts are placed inside a larger test tube, S, which is in turn placed inside a still larger test tube closed with a rubber stopper and of such size that it fits the centrifuge cup. The mouth of Q fits the ball of the filter rod well enough without grinding, so that the passage of any but the finest crystals will be prevented during centrifuging. The crystalline material will thus be caught at the mouth of Q. After centrifuging, Q and R can be lifted out together with a narrow pair of curved forceps and the mother liquor left in S. This apparatus has the advantage of affording small surfaces without the danger of fritted glass or shreds of filter paper.

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A New Oxidation-Reduction Reaction Catalyzed by Iodine

Application to the Detection of Iodide

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A new oxidation-reduction reaction catalyzed by iodine is described, by means of which as little as 0.2 microgram of iodide can be detected. The effect of 74 different anions and cations has been determined. A procedure for the detection of 0.03 mg. of iodide per ml. of solution in the presence of 50 mg. of interfering anion or cation has been developed.

The nitrite-arsenite reaction is catalyzed by other substances, but iodine is the most effective catalyst.

DURING the development of new methods for the systematic detection of the halides without the use of silver ion as a group reagent, and using lead and mercuric ions to remove interferences (*4*), a new oxidation-reduction reaction catalyzed by iodine was discovered. When nitrite was added to a dilute nitric acid solution containing lead arsenite in the presence of iodide ion, a heavy precipitate of lead arsenate formed; in the absence of iodide, no precipitate was obtained.

Bailey (*1*) reported that "dilute nitrous acid and arsenious acid react, either not at all or with extreme slowness in the presence of highly dilute sulphuric acid at ordinary temperatures".

Klemenc (*5*) developed a method for the determination of nitrous and arsenious acids in the presence of one another. Arsenious acid is titrated with iodine in the presence of an excess of sodium bicarbonate and then both the arsenite and nitrite are titrated in strong sulfuric acid with potassium permanganate.

Shilov and Perzner (*9*), in their attempt to oxidize salts of arsenious acid by the oxygen of the air, reported that neither nitrogen tetroxide nor iodine is effective as a catalyst. However, they found that a combination of hydrogen iodide and nitrogen oxides as catalysts effected complete oxidation of arsenious acid by air.

Baines (*2*) described a method for "the determination of small quantities of iodide in mixtures of halides depending on the pseudo-catalysis by iodide of the oxidation of thiosulfate by nitrous acid".

This new nitrite-arsenite reaction appears to be somewhat similar to the ceric-arsenite reaction (*3*, *6*, *7*) used as a basis for a method of detecting and determining small amounts of iodine. The ionic partials for the half cells in this reaction may be formulated as follows:

$$NO + 2H_2O \rightleftharpoons HNO_2 + H_3O^+ + e$$
$$HAsO_2 + 4H_2O \rightleftharpoons H_3AsO_4 + 2H_3O^+ + 2e$$

Although the molal oxidation potentials of nitrite and arsenite for the above half cells are -0.99 and -0.559, respectively (*8*), no evidence of any reaction between these two in acid medium was found after a search of the literature. According to Swift (*11*) arsenite can be oxidized to arsenate by boiling with sodium nitrite in a solution alkaline with ammonia. However, repeated attempts by the authors to effect this oxidation under the conditions described by Swift (*10*) were unsuccessful. Attempts to effect oxidation in acid medium were also unsuccessful, except in the presence of catalysts as described below. The most effective catalyst is iodine. By this new nitrite-arsenite reaction, as little as 0.2 microgram of iodide (as potassium iodide) can be detected, using the following procedure in which the limit of concentration is 1 part per 500,000.

Treat 2 drops of 1 M sodium arsenite in a 5-ml. beaker with 7 drops of 1 M nitric acid and add 1 drop of 1 M lead nitrate. Mix thoroughly and add the iodide (0.1 ml.). Now add 1 drop of 6 M sodium nitrite and let stand for 5 minutes, stirring occasionally. Add another drop of 6 M sodium nitrite and allow to stand for 10 minutes with occasional stirring. A white precipitate shows the presence of iodide. When less than 0.5 microgram of iodide is present, it is necessary to run a control without iodide, for on long standing a slight precipitate forms. However, this precipitate is exceedingly small when compared to that which is obtained when 0.2 microgram of iodide is present.

Applying this reaction on a macro scale, it was found necessary to modify the procedure as follows:

In a test tube treat 1 ml. of solution containing the iodide with 1 ml. of 1 M lead nitrate. Add 1 ml. of 6 M nitric acid, 1 ml. of 1 M sodium arsenite, and shake thoroughly. Then add 0.5 ml. of 6 M sodium nitrite, let stand for 1 minute, add another 0.5 ml. of nitrite, and let stand for 3 minutes. The formation of a heavy white crystalline precipitate shows the presence of

iodide. To ascertain the chemical composition of this precipitate, the lead was determined as lead sulfate. The percentage of lead was found to be the same as in $PbHAsO_4$. This procedure is capable of detecting 0.004 mg. or 4 micrograms of iodide.

Free iodine in aqueous solution also catalyzes this reaction.

Although the above procedures involving the formation of a white precipitate of lead arsenate are accurate and effective, it was deemed advisable to devise a procedure in which a characteristic colored precipitate for arsenate was obtained. Consequently, the following method involving the formation of silver arsenate was developed.

Treat 1 ml. of solution containing the iodide in a test tube with 1 ml. of water and 1 ml. of 6 *M* nitric acid. Add 1 ml. of 1 *M* sodium arsenite and mix thoroughly. Now add 1 ml. of 6 *M* sodium nitrite in 0.5-ml. portions at 1-minute intervals and after 3 minutes render the solution alkaline to litmus by the dropwise addition of 3 *M* sodium carbonate. Add 1 ml. of 0.5 *M* silver nitrate; the formation of a red-brown precipitate proves the presence of iodide. The lower limit of identification of this procedure is 0.01 mg. or 10 micrograms of iodide. This procedure may be modified by substituting 2 ml. of 1.5 *M* sulfuric acid for 1 ml. of water and 1 ml. of 6 *M* nitric acid.

Effect of Other Anions and Cations

The effect of other anions and cations upon the arsenite-nitrite reaction was determined by means of the following procedure:

Treat 1 ml. of solution containing 0.03 mg. of iodide in a 15-ml. centrifuge tube with 1 ml. of solution containing 50 mg. of anion or cation. Acidify the solution with 3 *M* nitric acid, add 1 ml. of *M* lead nitrate, and remove by centrifuging any precipitate that forms. Transfer the supernatant liquid to another centrifuge tube, add 1 ml. of 6 *M* nitric acid and 1 ml. of 1 *M* sodium arsenite, and thoroughly mix the solution. If any precipitate forms, remove it by centrifuging. To the supernatant liquid add 0.5 ml. of 6 *M* sodium nitrite. Let stand for 1 minute, add another 0.5 ml. of nitrite, and let stand for 3 minutes. The formation of a heavy white crystalline precipitate indicates the presence of iodide.

The following anions and cations cause no interference: bromide, chloride, ferricyanide, ferrocyanide, sulfite, oxalate, tartrate, phosphate, borate, sulfate, arsenate, chromate, perchlorate, cobalt, cadmium, manganese, nickel, magnesium, barium, strontium, zinc, calcium, tungsten, zirconium, uranium, bismuth, potassium, copper, ammonium, aluminum, lanthanum, erbium antimony, rubidium, cesium, platinum, osmium, thorium, rhodium, tellurium, vanadium, titanium, cerium (ous and ic), selenium (ous and ic), molybdenum, lithium, yttrium, and praseodymium.

In the presence of any of the following ions; thiocyanate, tin (ous and ic), chlorate, permanganate, iodate, bromate, ferrous, periodate, thiosulfate, gold, vanadium, and hydrogen peroxide, a precipitate is obtained even though iodide is absent. A gelatinous precipitate is obtained in the presence of stannous or stannic tin. With gold a chocolate-brown precipitate forms and with vanadium a yellow one. In the presence of iodide, stannous and stannic tin and gold yield the same type of precipitate as was mentioned above. With vanadium, however, the precipitate is white and crystalline as usual. When thiocyanate or thiosulfate are present together with iodide, the precipitate is not so heavy as is ordinarily obtained.

The least amount of hydrogen peroxide that will catalyze this reaction is about 21 mg. or 0.7 ml. of 3 per cent hydrogen peroxide. The lower limit of iodate and periodate is 200 and 300 micrograms, respectively.

With cyanide, sulfide, chromic, silver, fluoride, persulfate, mercury (ous and ic), ferric, beryllium, iridium, and ruthenium ions, no precipitate is obtained in either the presence or absence of iodide.

With stannous and stannic, ferrous and ferric, mercurous and mercuric, and chromic ions, the procedure was applied to a sodium carbonate extract prepared by boiling the sample with 1.5 *M* sodium carbonate. Where iron or chromium was present, 50 mg. of tartrate were added. Positive results were obtained when iodide was present and negative results when iodide was absent with stannic, ferric and ferrous (gelatinous precipitate), and chromium. With stannous ion a gelatinous precipitate formed in the absence of iodide, but with iodide the precipitate was white and crystalline. Mercury continued to inhibit the reaction under these conditions.

The interferences caused by fluoride, beryllium, thiocyanate, cyanide, sulfide, thiosulfate, chlorate, and bromate ions are removed by methods described below.

Procedure

On the basis of these experiments the authors have developed a procedure which is capable of detecting 0.03 mg. of iodide in 1 ml. of solution in the presence of 50 mg. of interfering anion or cation. This procedure does not apply in the presence of mercury, silver, iodate, periodate, hydrogen peroxide, permanganate, persulfate, gold, iridium, and ruthenium.

Treat 1 ml. of prepared sodium carbonate extract in a 15-ml. centrifuge tube with 3 *M* nitric acid until no more carbon dioxide bubbles are given off. Remove any precipitate that forms, add 1 ml. of 1 *M* lead nitrate, and centrifuge. To the supernatant liquid add 1 ml. of 6 *M* nitric acid and 1 ml. of 1 *M* sodium arsenite. Remove any precipitate that forms, add 0.5 ml. of 6 *M* sodium nitrite, and let stand for 1 minute. Add another 0.5 ml. of nitrite and let stand 3 minutes. A heavy white crystalline precipitate proves the presence of iodide.

NOTES. If fluoride is present add 3 ml. instead of 1 ml. of 1 *M* lead nitrate.

If beryllium is present add 1 ml. of 6 *M* sodium nitrite 1 minute after the addition of the second 0.5 ml. of nitrite and let stand 3 minutes.

If ferrous ion is present the precipitate although heavy will be gelatinous.

In the presence of thiocyanate 1 ml. of 0.3 *M* ferric nitrate must be added before the addition of 1 ml. of 6 *M* nitric acid.

If cyanide and sulfide are present treat 1 ml. of prepared solution in a 50-ml. beaker with 6 *M* acetic acid until no more carbon dioxide bubbles are given off. Add 0.5 ml. in excess and dilute to 6 ml. Evaporate over a small flame to 2 ml., cool, and apply procedure.

Use 6 *M* acetic acid instead of 3 *M* nitric acid if thiosulfate is present.

If chlorate and bromate are present, add 1 ml. of 6 *M* sodium nitrite before adding the arsenite and then add the nitrite in 0.5-ml. portions as usual.

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Correction

In the article entitled "Removal of Static Charges from Glassware by Ultraviolet Light" [IND. ENG. CHEM., Anal. Ed., 12, 693 (1940)], the temperature in Table I was erroneously given as ° C. whereas it should have been ° F.

CLEMENT J. RODDEN

Gasometer for Micro-Dumas Determinations

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THE use of a gasometer in the micro-Dumas determination for standardizing the blank and enabling the analyst to establish more accurately the end of the determination has been introduced by Niederl and co-workers (*3*, *4*). Mercury is utilized as the confining liquid. In this laboratory (*1*) carbon dioxide is supplied by a system in which the gas is stored over water. It was thought that a measuring apparatus employing the same confining liquid would be more satisfactory.

Such an apparatus is described below. It has the added advantage of being always under carbon dioxide pressure of the main gas supply. Once set, the gasometer is always ready to deliver a measured volume of carbon dioxide, since one chamber is automatically filled at all times.

Description of Apparatus

The apparatus is constructed of two 32 × 200 mm. test tubes, two three-way, 120°, elbow-bore stopcocks (*C* and *D*), a piece of glass tubing, and two three-hole rubber stoppers, as shown in Figure 1. The lower test tube is filled about three-fourths full with boiled water, the apparatus is assembled, and the stoppers are lacquered.

Method of Procedure

Air is flushed from the system by allowing carbon dioxide to flow through the apparatus with the stopcocks first in positions 1 and 2, then in positions 3 and 4. Flushing can also be accomplished by alternately filling and emptying each chamber as in actual operation. Once flushed adequately, the apparatus need not be flushed again.

As shown in the figure, the upper tube is ready to deliver the measured volume of gas. With the stopcocks in positions 1 and 3, gas is permitted to flow from the carbon dioxide supply, forcing the water in the lower tube from level *A* to level *B*. There is a corresponding movement of the water in the upper tube from *B'* to *A'*, displacing the measured volume of gas. The distance *AB* is marked on either the upper or lower tube (or both), and represents the desired amount of gas (ca. 50 ml.).

The next measured volume of gas is taken from the lower tube (which has become charged during the emptying of the upper tube) by turning the stopcocks to positions 2 and 4. During this operation the upper tube has become charged. An intermediate position of either stopcock serves to stop the flow of gas completely. In an idle period, stopcock *C* should be closed to prevent diffusion of air into the gasometer.

For the complete standardization of the Dumas method it was decided that not only the flushing period during the actual determination should be fixed, but also the initial flushing prior to combustion. Since the gasometer is used principally to avoid the necessity of judging the size of poorly defined "microbubbles" whose size varies according to the bore of the inlet tube of the azotometer (*5*), we should depend on it also for the initial flushing.

After the introduction of the sample, 1.5 measures of gas (75 ml.) are sent through the cold combustion tube to discharge the air. The tube is heated for 2 minutes while part of the remaining half measure is slowly passing into the azotometer. Excess carbon dioxide from this half measure is discharged into the atmosphere, except for the last few milliliters, which are sent into the azotometer. Stopcock *C* is closed (by turning to an intermediate position), the azotometer is refilled, and the combustion is started.

In this manner the error is incorporated entirely in the blank.

TABLE I. ABSOLUTE BLANK DETERMINATIONS

	Observed	Calculated	Difference −0.8%	Absolute Blank
Hippuric acid	0.583	0.563	0.020 − 0.005	0.015
	0.522	0.505	0.017 − 0.004	0.013
	0.488	0.470	0.018 − 0.004	0.014
	0.418	0.400	0.018 − 0.003	0.015
Acetanilide	0.776	0.755	0.021 − 0.006	0.015
	0.542	0.524	0.018 − 0.004	0.014
	0.431	0.403	0.018 − 0.003	0.015
	0.345	0.327	0.018 − 0.003	0.015
			Av.	0.0145

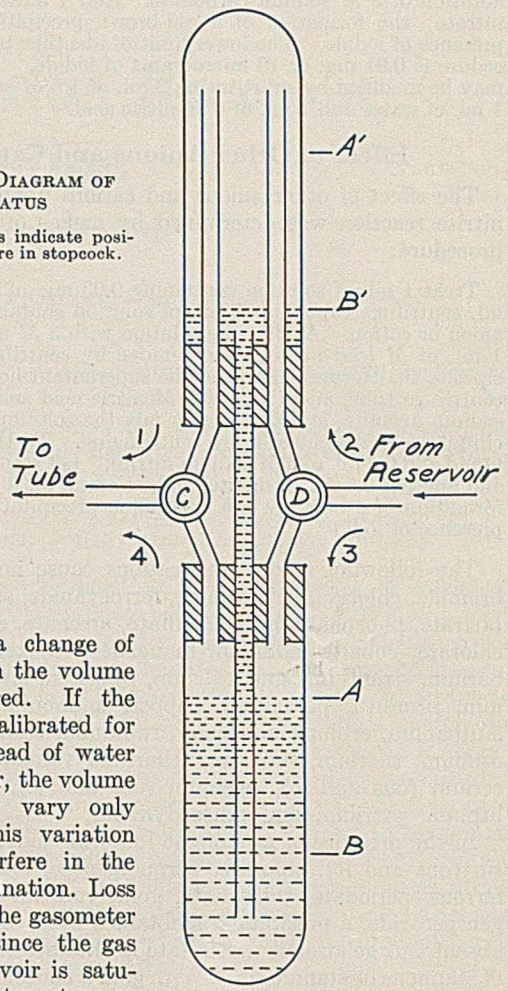
Discussion

Gas is delivered from the measuring apparatus at approximately the same pressure as if it were delivered directly from the reservoir.

Since the system is completely closed to the atmosphere, water can be used as the confining liquid. A leveling bulb is therefore unnecessary, because the gas is never supporting more than about 60 cm. (2 feet) of water (in the reservoir). The change in pressure during emptying of the reservoir then causes a change of about 3 ml. in the volume of gas delivered. If the apparatus is calibrated for the average head of water in the reservoir, the volume delivered will vary only ±1.5 ml. This variation does not interfere in the Dumas determination. Loss of water from the gasometer is negligible, since the gas from the reservoir is saturated with water at room temperature.

FIGURE 1. DIAGRAM OF APPARATUS

Numbered arrows indicate position of elbow bore in stopcock.



The measuring apparatus has been in use in this laboratory for the past year. Absolute blank determinations, made as indicated by Niederl (*2*), give an average of 0.014 ± 0.001 ml. (five determinations).

This blank is confirmed by actual test runs on very pure substances. After subtracting the corrections for vapor pressure and adhesion of potassium hydroxide (0.8 per cent, *2*), the difference between the observed and calculated volume of nitrogen represents the absolute blank. Results obtained from analysis of hippuric acid and acetanilide are given in Table I.

Acknowledgment

The authors wish to thank Francis W. Power, S. J., for help and advice in this work.

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Adapting Polarizing Microscope for Use as a Polarimeter

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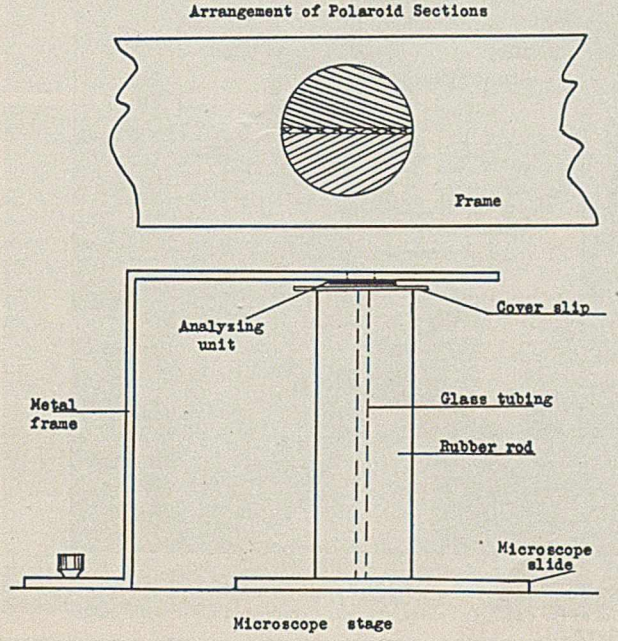
THE polarizing microscope can be adapted for use as a polarimeter by the addition of a simple analyzer constructed from a few square centimeters of Polaroid. The addition of such an analyzer greatly increases the versatility of the polarizing microscope, which is a more common laboratory instrument than the polarimeter. A primary advantage of the microscopic method is the small quantity of sample which will suffice to fill the specimen tube, only 150 cu. mm. being necessary.

The analyzing unit consists of a metal frame which can be attached firmly to the graduated stage by means of a knurled machine screw, ordinarily used in fastening a mechanical or Federoff stage to the instrument. The height of the frame must be selected so as barely to clear the top of the cell.

The two small pieces of Polaroid are located beneath a hole drilled in the metal frame concentric with the optical axis of the microscope. The sections of Polaroid are cut so that their planes of polarization include an angle of approximately 5° when the segments are mounted in place with a slight overlap.

The cell is essentially a length of 2-mm. glass tubing cemented in a hard-rubber rod and then fastened to a microscope slide for easy manipulation. The height is selected so that the rack and pinion gears of the microscope adjustment are engaged and allow focusing, taking into consideration the thickness of the cover glass which is on top.

The frame is bent from a strip of aluminum (2.5 cm. wide), so that the distance between the microscope stage and the top of the frame is 5.25 cm. This provides sufficient clearance for the cell which is 5.15 cm. overall and has an effective cell length of 5.0 cm. A 5-mm. hole is bored in the frame and the Polaroid fastened beneath.



In use, the microscope is focused upon the slightly overlapping intersection of the two pieces of Polaroid. This junction should approximately bisect the field and when the stage is rotated the mid-point of the intersection should remain in the center of the field. Coupled with the manner of mounting of the Polaroid, this procedure gives a field roughly halved, in which the intensity of the light will be uniform only at the zero point.

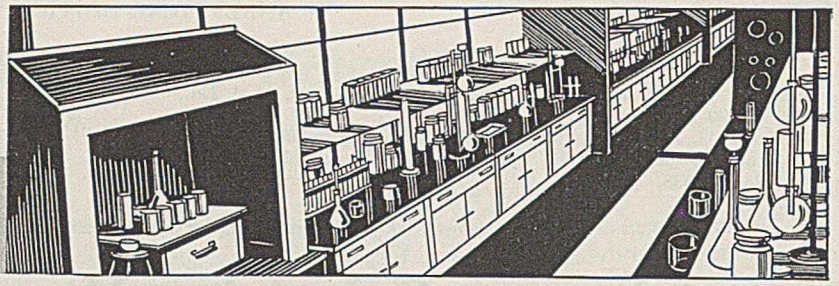
A magnification of × 100 is sufficient to make the end of the tubing cover the entire field. Higher magnification serves no useful purpose, since it complicates the adjusting of the microscope and does not produce any refinement in procedure. Best results were obtained when a compromise plane of focusing was selected midway between the Polaroid and the top level of the liquid in the cell. This caused the junction of the polarizing films to become indistinct but brought the end of the cell more closely into focus. It is for this reason that it is recommended that the metal frame be constructed to be as close as possible to the top of the cell, allowing for the cover glass, and that the Polaroid be mounted on the underside of the frame.

TABLE I. RESULTS OBTAINED WITH APPARATUS

	Calculated	Observed	Error, %
Dextrose, 25%	6° 39′	6° 32′	1.8
Dextrose, 16.7%	4° 25′	4° 22′	1.1
Dextrose, 12.5%	3° 18′	3° 1′	8.5
Dextrose, 8.3%	2° 12′	2° 15′	2.3
Levulose, 25%	11° 30′	11° 48′	2.6
Maltose, 25%	17° 15′	16° 19′	5.4
Maltose, 25%	17° 15′	16° 18′	5.4
Maltose, 12.5%	8° 38′	8° 48′	1.9
Sucrose, 25%	10° 25′	10° 43′	2.9

The solution can be made up in a 1-ml. volumetric flask by weighing out 100 to 250 mg. of the solid on the analytical balance and dissolving in sufficient water to make the proper volume. To transfer the solution, a portion is sucked up in a capillary tube, the tip of which is then placed in contact with the bottom of the cell, and the liquid is carefully expelled. As the cell is filled, the capillary is slowly withdrawn, always keeping the tip below the surface of the liquid. Advantage is taken of surface tension to create a hill of liquid above the top of the cell. The cover glass is slipped on top and the excess liquid absorbed by a piece of filter paper. This draws the cover glass tightly against the top of the cell and also ensures the complete filling of the cell. It is important to obtain the volume of liquid entirely free of air bubbles; a suitable check is to hold the cell directly at a light, when a translucent disk of light free of any dark spots will indicate complete filling.

To obtain the zero point, the cell, filled with distilled water, was manipulated until it was in the field of view and the stage rotated until both halves of the field were equally extinct. Monochromatic light was obtained from a sodium vapor lamp; the intensity of this light was regulated by the substage iris until the zero point illumination was sufficient to provide a sharp zero point. A simplification in procedure resulted when the zero point found using the water cell was identical to that determined without any cell. This suggested the feasibility of dispensing with the cell in locating the zero point. By this method the cell need be filled only once with the unknown solution, and dilution errors will be minimized.



MODERN LABORATORIES

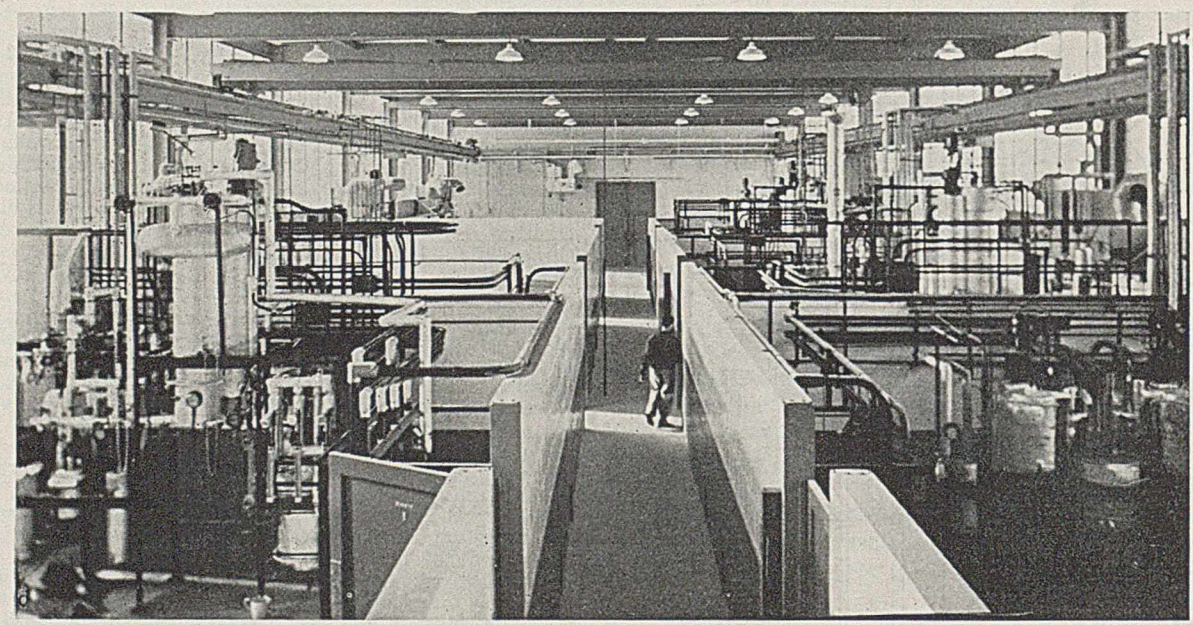
Houdry Laboratories of the Catalytic Development Company

H. P. BROOM, Catalytic Development Company, Marcus Hook, Penna.

THE Catalytic Development Company, developer of the Houdry process for the catalytic conversion of petroleum fractions, has recently completed a laboratory installation at Linwood, near Marcus Hook, Penna., designed to provide facilities for fundamental physico-chemical catalytic investigations, including small-scale preparation and testing of experimental catalysts; study of catalytic processes in pilot units essentially duplicating commercial installations, and associated chemical engineering problems; and analytical and testing laboratories and shops. There are one building housing research and test laboratories and offices, a development laboratory, a shop, and a sample storage building. A knock test laboratory is under construction. The plot of 7 acres allows for expansion of the research and test laboratory building, duplication of the development laboratory building, and extension of the shop and sample storage facilities. Particular attention has been paid to obtaining ample window space in all buildings. Total floor area is approximately 35,630 square feet.

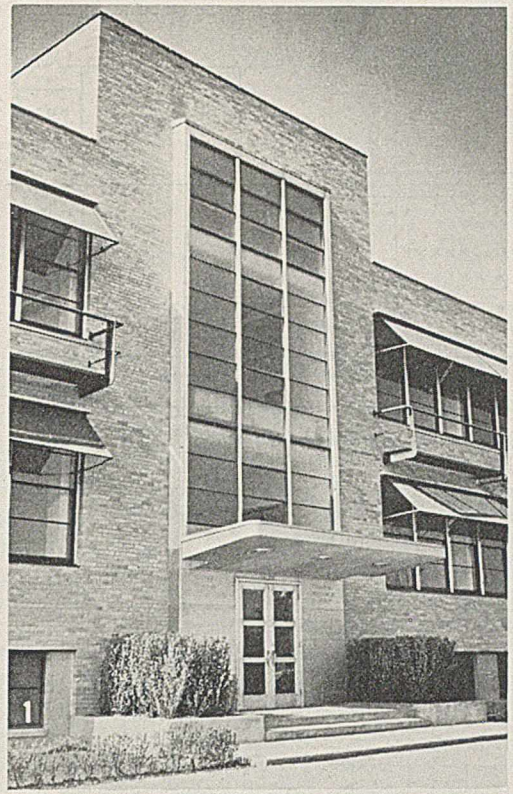
The entrance to the research and test laboratory building is emphasized by an aluminum and glass panel extending from the ground to an equipment penthouse above the second story. In the entrance lobby, which is modernly utilitarian in design, the telephone operator serves as a receptionist. In the short wing to the left on the first floor is a general office, 40 × 25-feet, for maintenance engineer, purchasing agent, stenographers, etc., which is equipped with an acoustic ceiling. To the right are six offices occupied by supervisory engineers and liaison men with other departments of the company. The right-hand half of the ground floor is the test laboratory where conventional petroleum inspections are carried out. Improved controlled reflux distillation towers are here installed, in place of the Hempel type, for the separation of gasoline from synthetic crude in quantities sufficiently large for testing. Two of the latest design Podbielniak Robot distillation columns are part of the test laboratory equipment.

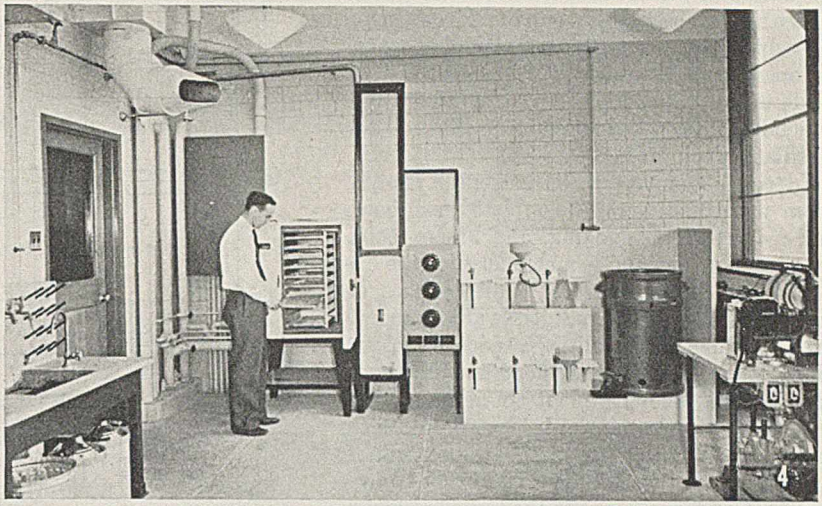
On the second floor are offices for the head of the laboratories division and of the research department, and the library, and research laboratories for organic analysis, inorganic analysis, preparation of experimental catalysts, routine testing of experimental catalysts, etc. In one room, assigned to distillation, are a 100-plate column still and several stills of 25 to

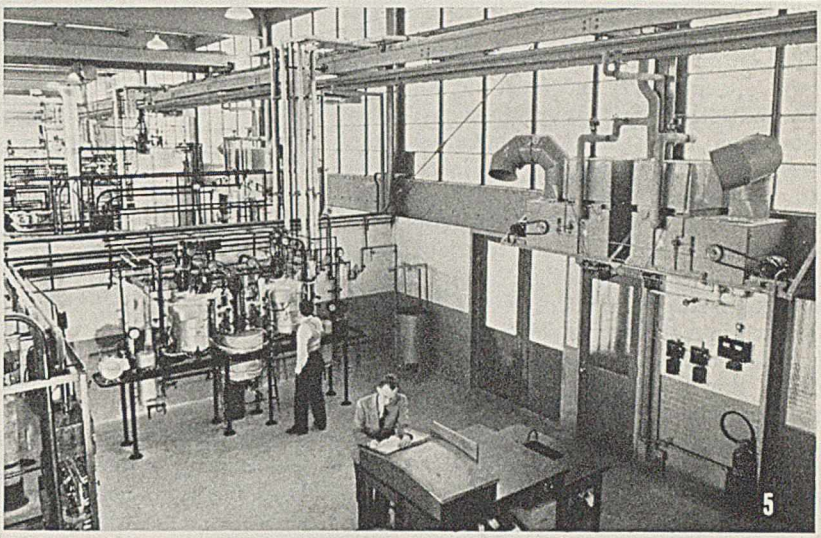


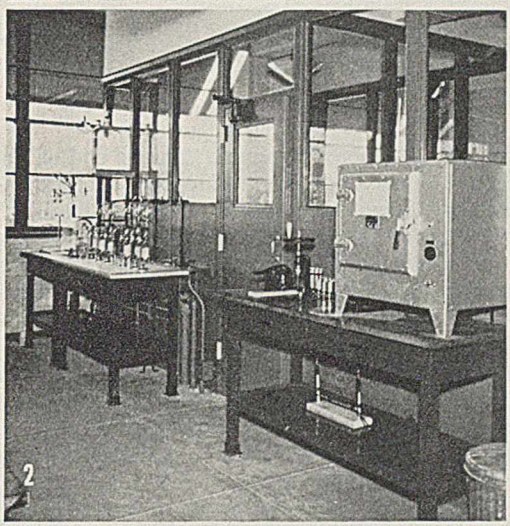
INTERIOR OF DEVELOPMENT LABORATORIES

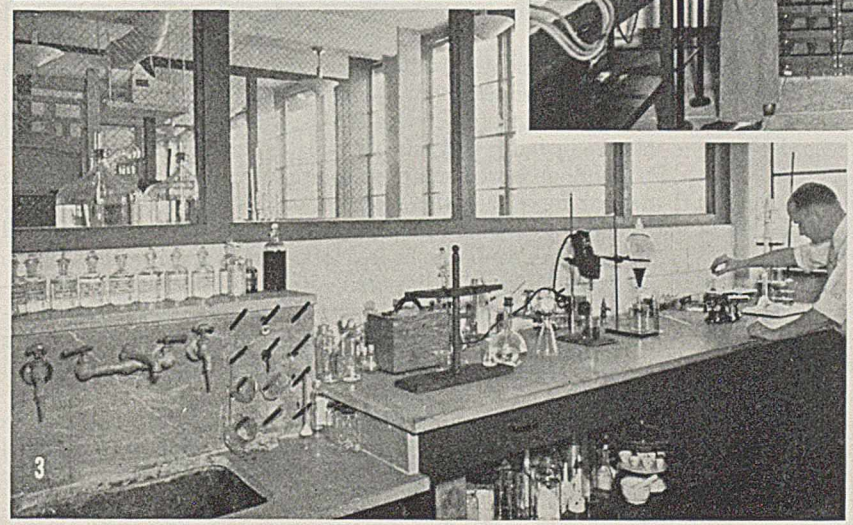
General arrangement of six laboratories, three on each side, with large room at far end. In center, traveling crane and hoist with guide chain hanging down.











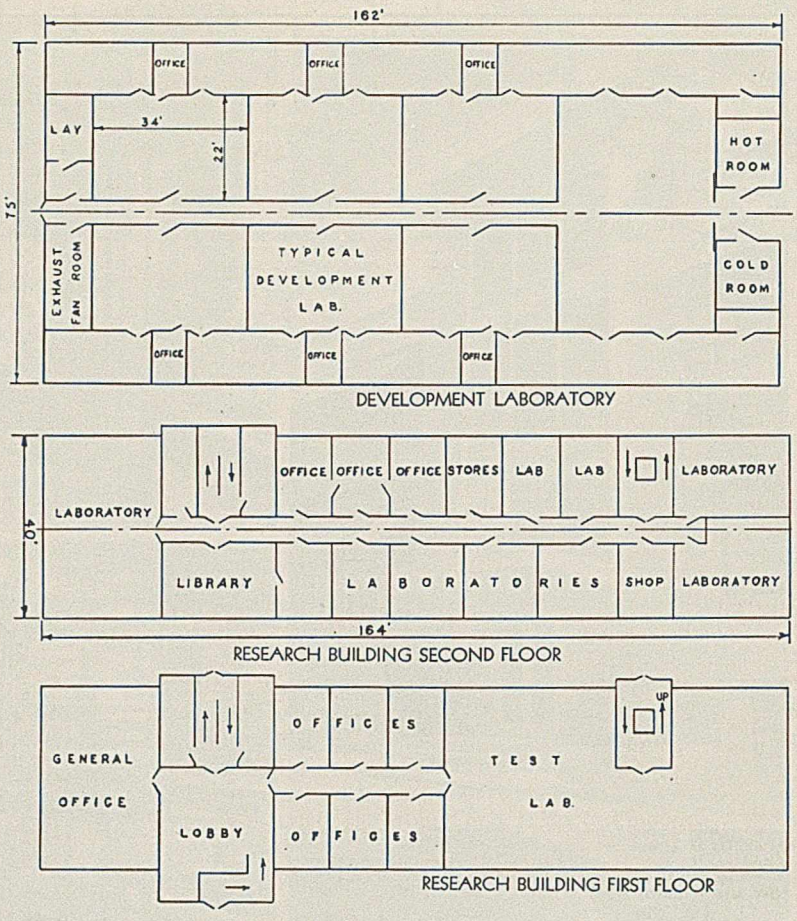
Development and Test Laboratories

1. Entrance to building. 2. Section of test laboratories, showing lamp sulfur setup, door to balance room, cork softener and borers, and oven. Enclosed flash and fire room behind oven. 3. One of smaller research laboratories. 4. Research laboratory, showing man at pan dryer, high-temperature electric furnace, rack for filtering and mixing, and small ball mill. 5. Development laboratory. Control panel (back view) at extreme left, pilot unit with operator, ventilating equipment above office door. 6. Shop in research laboratory. Hoses of glass blower on left. Man operating small drill press. Small lathe in center, finished glassware, tool rack, and vise at right

30 plates, all with controllable variable reflux. There are also a small storeroom and a shop for glass blowing and small-scale machine work. In one of the laboratories a hole in the floor leads to an open spot in the test laboratory which has already been used for packing of tall distillation columns.

In the basement are 85 lockers, a shower room with 6 showers, a complete washroom, and a recreation, smoking, and lunch room. In a room at one end is machinery for the experimental production of small batches of catalyst. A storeroom, glassware laundry, print machine, and utilities service room are along one side. The first floor is well above ground level, allowing plenty of light to enter the basement. A stair well is located near each end of the building; in one is a dumb waiter for moving heavy cylinders and other equipment from the basement to the test or research laboratory floors.

The entire building is ventilated by forced draft. Recirculated and fresh air is filtered and sent to the offices; it may be cooled by the installation of a simple refrigeration unit. Fresh air is blown into all the laboratories and exhausted through the hoods to the outside. Flake ice is produced by a 1-ton-per-day refrigeration machine in the test laboratory and distributed to the other laboratories in insulated boxes as needed. Distilled water is prepared in a still in the penthouse above the second floor and piped to the analytical laboratories. Small carbon dioxide fire extinguishers are in each laboratory and larger ones are in the halls. Emergency showers are placed by the door of each laboratory and fire blankets are hung on the walls. A steel balcony runs around the outside of the second floor, providing a means of escape in case of fire in one of the laboratories between a man and the door. All laboratory doors open out to the halls.



The development laboratory is of industrial type construction with a flat roof 21 feet high. It is divided by walls 8 feet high into six sections 35 × 22 feet, each housing two or three pilot units, and one larger section 50 × 35 feet for larger scale chemical engineering equipment. An office connecting with each development laboratory provides desk space for two development division engineers. The pilot units are all steel, operate at commercial cracking temperatures and pressures, and have throughputs ranging from 1 to 20 gallons per hour. A table and sink in each laboratory provide a place for record notebooks, weighing and measuring charge and products, etc. Room is left behind all pilot units for making repairs easily. Control panel boards are an integral part of each unit with switches mounted for electrical heat control as well as temperature and pressure recorders and indicators.

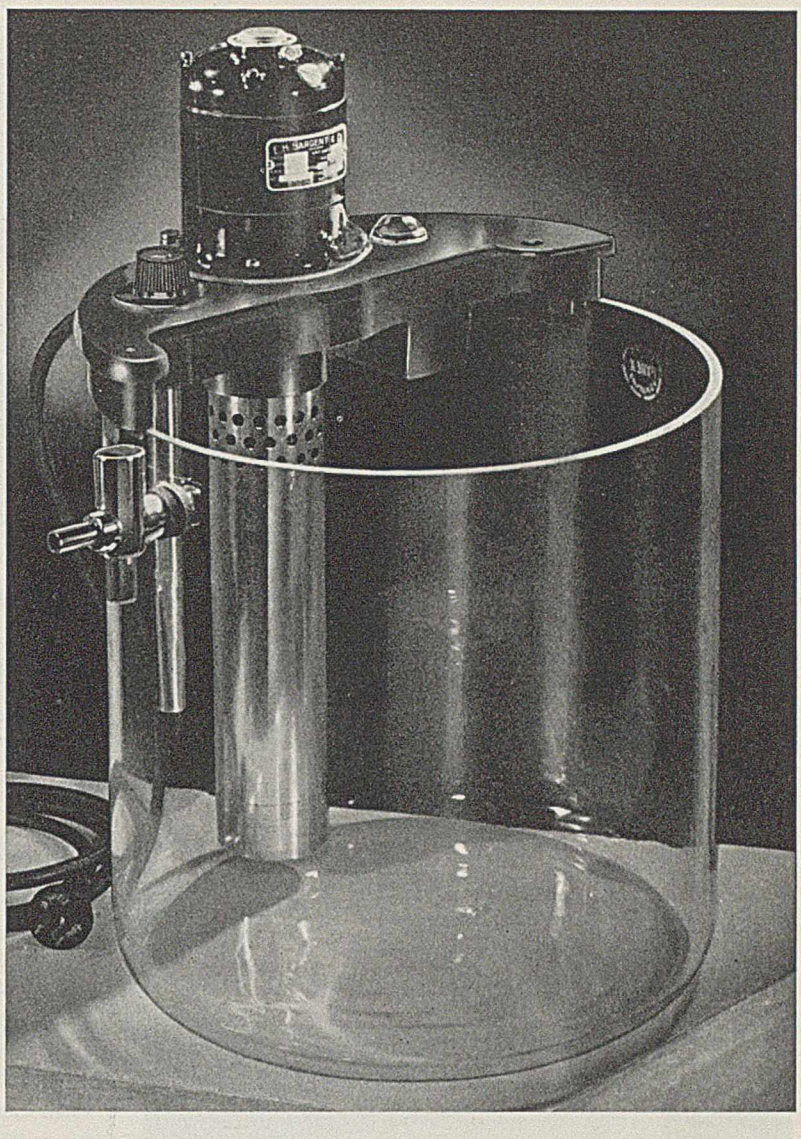
No container of oil over 5 gallons' capacity is permitted inside the laboratory; a porch runs along each side of the building to cover drums and larger auxiliary equipment from which oil can be pumped to the pilot units. In the roof over the large room are four panels 4 × 5.5 feet to permit running towers or other tall equipment up through the roof. A truck can be backed into this room and a 2-ton crane can pick up equipment from the truck and deliver it to any spot in the laboratory. There are 18 feet of headroom under the crane.

Ventilating and heating are by 16 blowers spaced around the walls of the laboratory. Air is exhausted by 8 fans in the roof and by 16 outlets to a duct in the floor. In this way the accumulation of hazardous heavy vapors is avoided and hot air rising to the roof is removed. At one end of the development laboratory is a hot room where, by use of a cabinetlike arrangement, drums of heavy oil are kept at 150° F. while the rest of the room is not uncomfortably warm. Across from the hot room light oil samples are kept at 50° F. by a unit which also provides 50° F. cold water around the laboratory for uniform condensing conditions. All the development laboratory equipment is electrically heated, and 4000 amperes at 110 volts are distributed through a copper bar "Buss-Wa" system which may be tapped at any point around the laboratory. A central vacuum system operated by a steam jet gives a dependable vacuum equal to the best that can be obtained in normal commercial installations. No house vacuum is supplied in the research laboratory, it being felt that better results could be obtained by individual pumps.

The knock test laboratory will house two convertible C. F. R. engines to run either A. S. T. M. or '39 research method octane tests. An AFD-1C aviation test engine will also be installed and humidity-regulated air will be supplied.

The shop is well equipped to produce most of the apparatus needed for either the research or development laboratory. Machine tools and welding equipment are allotted about equal space. The shop also houses locker room and lavatory for the mechanical force, an instrument shop, storeroom, fire equipment, garage, electrical metering equipment, and air compressors. Steam is obtained at 450 pounds pressure from the Sun Oil Co. near by and a reducing valve for the steam supply is also in the shop.

The storage building is of Transite and is designed to store oil, catalyst, and miscellaneous samples. An adjacent roofed section provides storage for quantities of light oil drums, while heavy oil drums are stored in an adjoining uncovered area. An oil separator is provided for surface sewage.



A NEW SARGENT CONSTANT TEMPERATURE WATER BATH

Guaranteed accuracy and uniformity, ±0.25°C. All immersed vessels fully visible through walls of bath

This new bath has just two compact operating units—a linear expansion type thermoregulator and a heating and circulating tower. The illustration clearly shows how this concentration of parts leaves a high percentage of the total bath space entirely free of obstructions, and so materially increases the amount of space that can be used for insertion of vessels.

From the standpoint of convenience, there are fewer elements to assemble, adjust and care for. Both the thermoregulator and heating and circulating tower are integrally mounted on a one-piece lightweight aluminum casting which rests on the rim of the heavy Pyrex glass container. The entire unit can be lifted out of the bath when desired—there are no clamps of any kind.

Performance is guaranteed—accuracy of regulation and uniformity of temperature at *every* point in the bath are ±0.25°C. The circulating and heating tower contains a turbine impeller and all heating units. Heat is thus correctly applied to the water at the point of its most rapid circulation. The uniformity of temperature produced by this bath is directly dependent upon this type of heating and circulating system which is the same as that used in the 0.01° Sargent Constant Temperature Water Bath.

S-84860 WATER BATH—Constant Temperature, Full Visibility, 0.25°C., Sargent. Complete with heavy molded Pyrex glass container, 12 inches high and 12 inches in diameter; circulating and heating unit with motor; linear expansion type thermoregulator with temperature reference dial, and cord and plug. Water depth, 11 inches. For operation from 110 volt 60 cycle single phase circuits..... $75.00

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