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Sulfur Train 418

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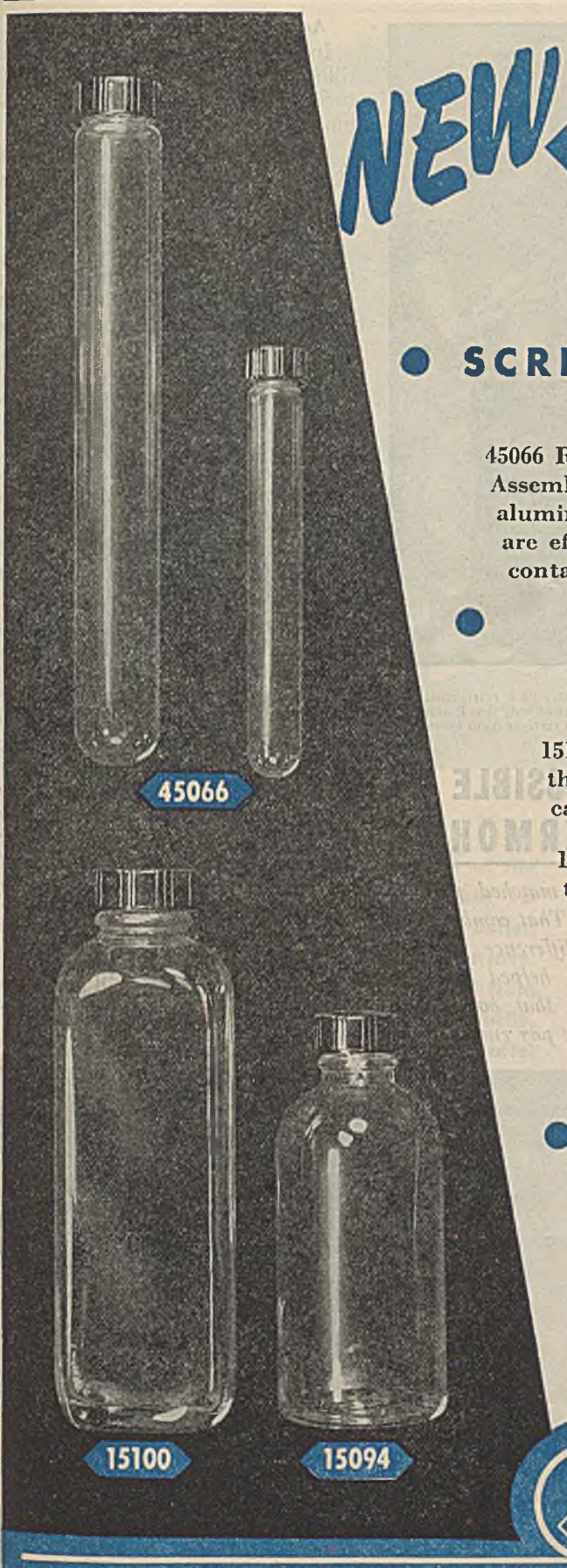

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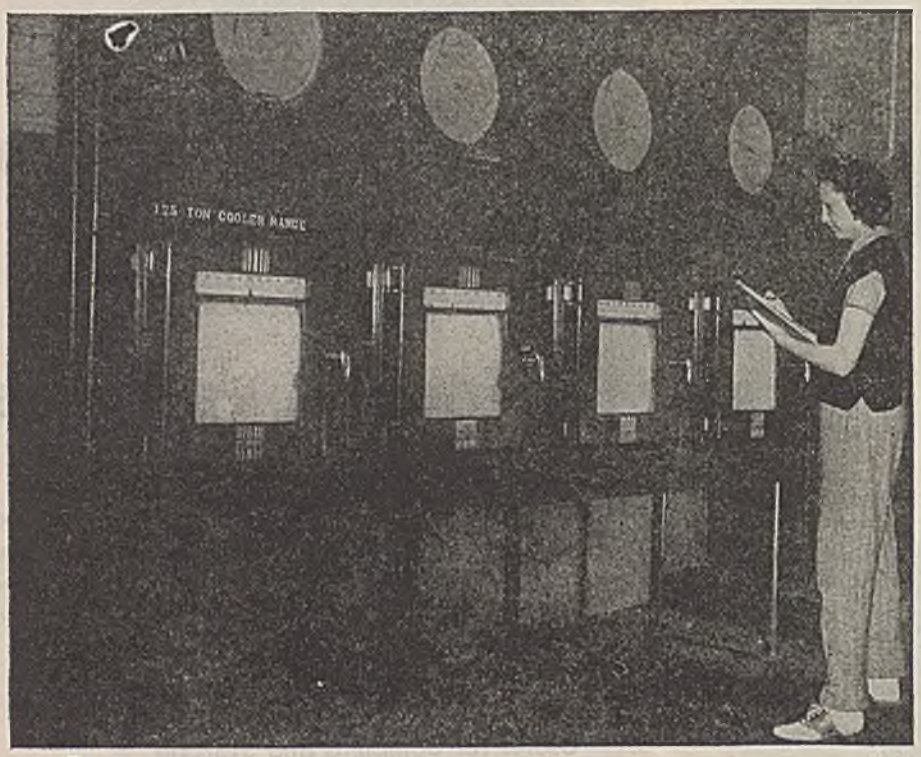

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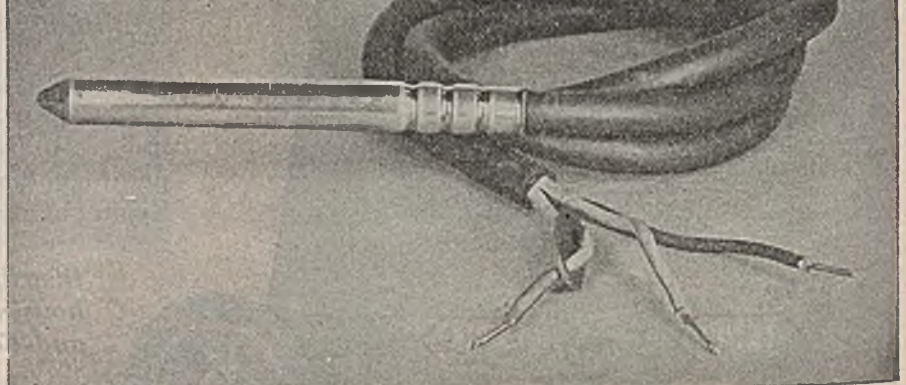
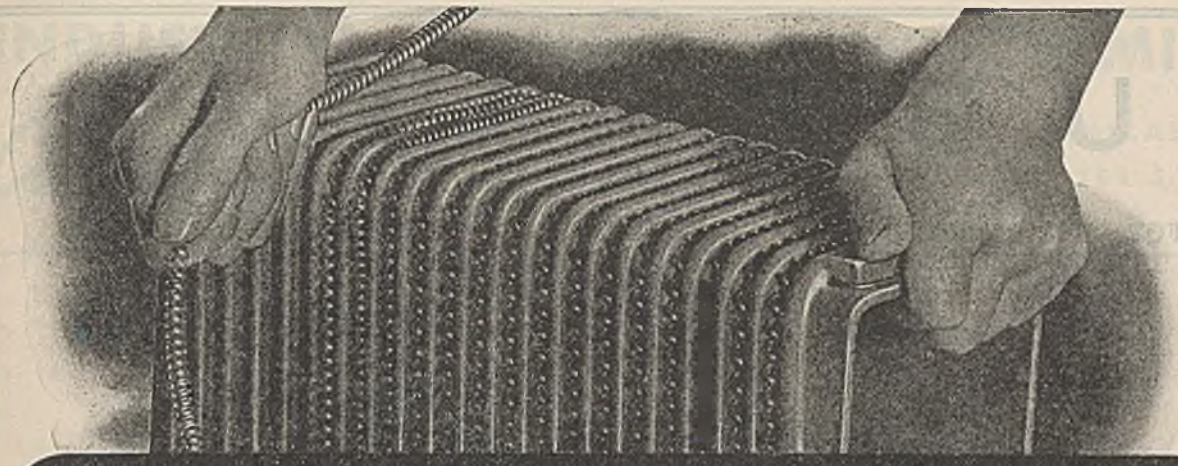


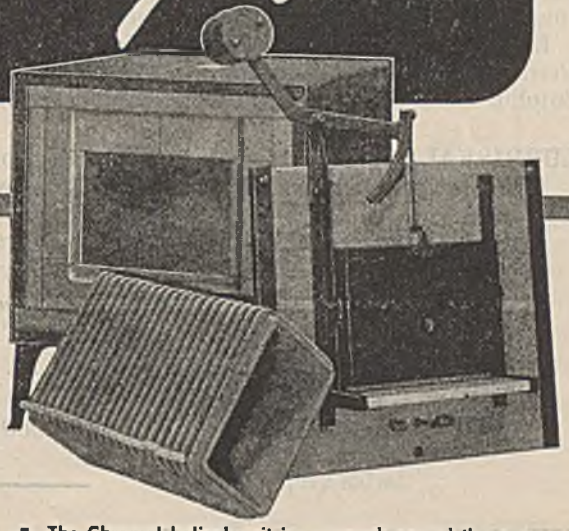
Thermohm temperature-detector; simply expose it to temperature and run its leadwires to the Micromax Recorder. If it corrodes or is broken, just replace it; Micromax calibration isn't affected.

Jrl Ad N-33C-700(1)



A FEW TWISTS OF THE WRISTS

- and all set to go!



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Molecular Weight of Cellulose

Measurement of Average Degree of Polymerization

O. A. BATTISTA, American Viscose Corporation, Marcus Hook, Pa.

Viscosity-concentration data are given for five samples of purified cellulose representing the degree of polymerization range from 300 to 3000. On plotting the data on semilogarithmic paper, linear relationships were found to exist, in each case, between (1) the viscosity function $\frac{\eta sp}{c}$ and concentration, and (2) the relative viscosity function measured at 0.5% concentration and the degrees of polymerization corresponding to values calculated from viscosity-concentration data extrapolated to infinite dilution. The data have been used to derive a mathematical expression by means of which the value of the viscosity function at the standard concentration of 0.5% may be converted to degree of polymerization data equivalent to values obtained by extrapolation of viscosity-concentration data to infinite dilution.

WITH the advent of the more rigorous concepts of cellulose as a long-chain molecule of high molecular weight, the deteriorating action of chemicals and heat on cellulose has come to be considered as a depolymerization reaction whereby the monomeric glucose anhydride units linked continuously in the cellulose chains become severed at irregular intervals in the chains, giving rise to shorter molecules.

The publication of the Staudinger (*11*, *12*, *13*) empirical viscosity–molecular weight relationship gave great impetus to the investigation of methods for the determination of the weight-average molecular weights of high polymeric compounds. Coppick (*2*) has recently reviewed and discussed the more significant papers that have been published relating the viscosity of solutions of high polymers with the degree of polymerization. The procedure most widely used is to relate viscosity data obtained at relatively high concentrations with the value of the viscosity function at infinite dilution through the use of mathematical equations (*2*, *4*, *5*, *6*, *8*, *14*).

In this paper, viscosity data are presented for five samples of purified cellulose representing the practical degree of polymerization range from 300 to 3000. These data illustrate that a linear semilogarithmic relationship exists between the relative viscosity measured at 0.5% concentration and the degree of polymerization calculated from viscosity data extrapolated to infinite dilution. The constants of the equation expressing this experimentally determined relationship have been obtained by a graphical analysis of the data, and using these constants the equation has been satisfactorily checked against extrapolated values.

EXPERIMENTAL

METHOD OF FLUIDITY MEASUREMENT. The general procedure used for the measurement of fluidity was based on the papers by Clibbens and Geake (*1*) and Mease (*9*). The viscometer's design, complete dimensional specifications, method of calibration, and a discussion of the precision of viscosity measurement obtainable with this type of capillary viscometer, are given in the foregoing papers.

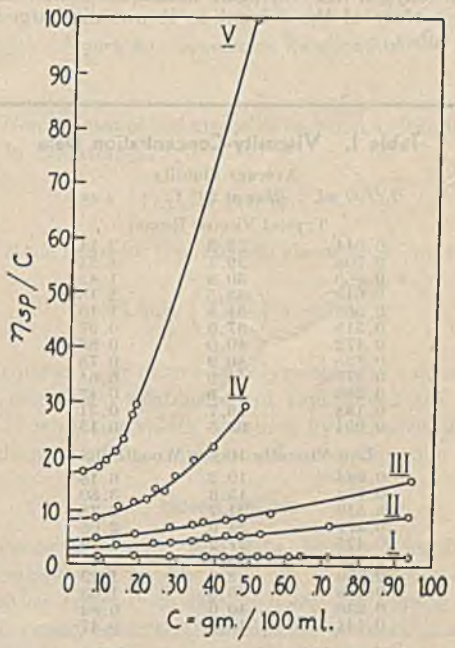

Figure 1. $\frac{\eta sp}{c}$ vs. c (ordinary graph paper)

I. Typical viscose rayon
II. Low-viscosity rayon wood pulp
III. Normal-viscosity rayon wood pulp
IV. Absorbent cotton
V. Raw cotton

The viscometers used in this work were equipped with ground-glass connections (*3*, *9*) and glass stopcocks. Outside dissolving tubes (*10*), whereby the viscometers are reserved for the measurement of fluidity, were used.

It was found advisable, in determining the viscosity of high-fluidity celluloselike rayon, to use viscometers possessing capillaries of smaller inside diameter than the 0.88-mm. inside diameter capillary recommended for use with cotton solutions. The large kinetic energy correction that would otherwise be necessary for high-fluidity cellulose solutions may be satisfactorily reduced by the use of viscometers whose capillaries have an inside diameter of 0.675 mm.

Pure copper gauze (80-mesh) was used in the preparation of the cuprammonium solvent. The copper gauze was wrapped around an inlet tube equipped with a fritted-glass jet of D porosity, and maintained below the level of the ammonium hydroxide in the generating chamber. Agitation was provided for by the fritted-glass jet which served to break up the incoming ammonia-laden air into small bubbles. The use of fine-mesh copper gauze facilitated the solution of the copper, and obviated any necessity for filtering the solvent at any time in the process

of its preparation. A siphon was used to transfer the solvent from the generating chamber to the stock bottle.

The copper content was determined by means of a calibrated photoelectric colorimeter. This method is rapid and was shown to be as accurate as the volumetric method for determining copper. The copper content was adjusted to 15.0 (±0.10) grams of copper per liter.

The ammonia content was determined volumetrically and was maintained at 200 (±5) grams of ammonia per liter.

The nitrous acid content was determined by means of a Lunge nitrometer and was never found to exceed the maximum limit of 0.5 gram per 100 ml. of solvent.

The solvent was stored under oxygen-free nitrogen at 5° C., and its viscosity in centipoises ranged from 1.32 to 1.36 at 20° C.

All samples of cellulose used in this study received a mild alkaline scouring treatment (1% sodium hydroxide at 40° C. for at least 40 minutes), and a thorough extraction with water and organic solvents. Samples were conditioned at 58% relative humidity and 21.11° C. (70° F.) for at least 24 hours, after which moisture determinations were made in duplicate on each sample. The weight of the sample used for the measurement of fluidity was calculated on a bone-dry basis.

Black glazed analytical weighing paper was used for weighing the samples.

The samples were put up in a constant-temperature room (18° C.), and left on the rotating wheel at this temperature overnight. An oxygen-free nitrogen atmosphere was maintained above the surface of the solvent as it was discharged into the dissolving tubes.

Table I. Viscosity-Concentration Data

c' %	c G./100 ml.	Average Fluidity Rhes at 20° C.	η_{sp}	$\frac{\eta_{sp}}{c}$
		Typical Viscose Rayon		
1.00	0.944	23.3	2.14	2.26
0.75	0.708	29.1	1.51	2.13
0.70	0.660	30.8	1.42	2.15
0.65	0.613	33.5	1.18	1.90
0.60	0.566	34.8	1.10	1.94
0.55	0.519	37.0	0.97	1.86
0.50	0.472	40.0	0.86	1.82
0.45	0.425	40.9	0.78	1.83
0.40	0.377	44.9	0.64	1.69
0.30	0.283	49.6	0.47	1.66
0.20	0.188	55.7	0.31	1.64
0.10	0.094	63.5	0.15	1.59
		Low-Viscosity Rayon Wood Pulp		
1.00	0.944	10.2	6.18	6.54
0.75	0.708	15.3	3.80	5.36
0.55	0.519	21.7	2.38	4.58
0.50	0.472	24.0	2.06	4.36
0.45	0.425	27.2	1.70	4.00
0.40	0.377	29.8	1.47	3.89
0.35	0.330	32.9	1.23	3.72
0.30	0.283	36.3	1.03	3.63
0.25	0.236	40.0	0.84	3.55
0.15	0.141	49.9	0.47	3.33
		Normal-Viscosity Rayon Wood Pulp		
1.00	0.944	5.56	12.2	12.81
0.60	0.566	14.0	4.24	7.49
0.50	0.472	17.5	3.18	6.73
0.45	0.425	19.6	2.76	6.40
0.42	0.395	21.3	2.45	6.18
0.40	0.377	22.8	2.22	5.88
0.38	0.358	22.9	2.21	6.17
0.35	0.330	26.5	1.78	5.39
0.30	0.283	30.4	1.42	5.02
0.20	0.188	39.9	0.87	4.62
0.10	0.094	52.9	0.39	4.14
		Absorbent Cotton		
0.50	0.472	5.19	13.3	28.17
0.40	0.377	8.28	7.88	20.90
0.35	0.330	10.3	6.14	18.60
0.30	0.282	13.6	4.43	15.75
0.25	0.236	17.4	3.24	13.72
0.23	0.217	19.1	2.91	13.40
0.22	0.208	21.0	2.55	12.25
0.20	0.188	22.5	2.33	12.30
0.15	0.141	28.5	1.62	11.50
0.10	0.094	38.8	0.925	9.78
		Raw Cotton		
0.50	0.472	1.45	48.0	101.6
0.25	0.236	7.38	8.98	38.05
0.20	0.188	11.4	5.42	28.61
0.18	0.169	13.9	4.28	25.32
0.15	0.141	18.3	3.03	21.49
0.12	0.113	22.6	2.23	19.72
0.11	0.104	24.9	1.95	18.75
0.10	0.094	27.1	1.75	18.61
0.09	0.085	29.8	1.50	17.65
0.08	0.075	31.5	1.37	18.35
0.05	0.047	43.2	0.73	15.55

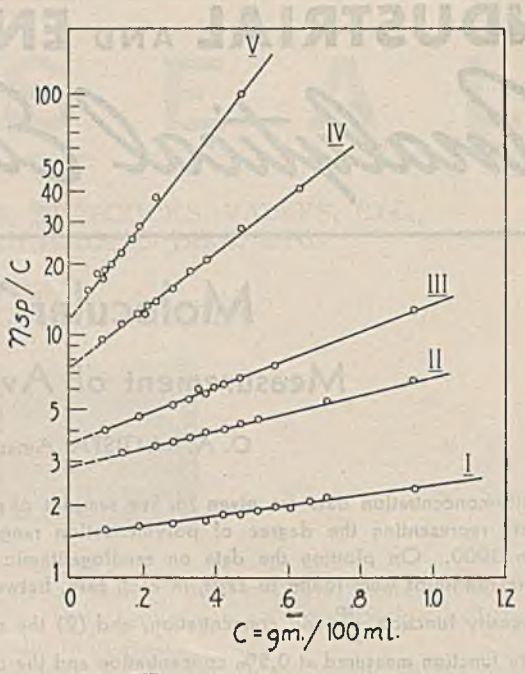

Figure 2. $\frac{\eta_{sp}}{c}$ vs. c (semilogarithmic graph paper)

I. Typical viscose rayon
II. Low-viscosity rayon wood pulp
III. Normal-viscosity rayon wood pulp
IV. Absorbent cotton
V. Raw cotton

Pure copper agitators in the form of spirals or solid rods, depending on the viscosity of the sample being tested, were used to minimize the degradative action of oxygen on cellulose in cuprammonium solution (3).

Fluidities were measured at 20° (±0.10°) C., and flow times were determined by means of a split-second electric stop clock, with an average reproducibility to within less than 1%. The average deviation in the fluidities of the duplicate measurements on a given sample never exceeded 5%, and was usually less than 2%.

A solvent blank was run in duplicate with each series of determinations. A standard sample of cellulose was run as a check blank periodically. New batches of solvent were prepared every 2 or 3 months and 3 liters were prepared at a time.

RESULTS

In Table I viscosity-concentration data are given for each of the five samples of cellulose studied: a typical viscose rayon, a low-viscosity rayon wood pulp, a normal-viscosity rayon wood pulp, absorbent cotton, and raw cotton.

In Table II, degree of polymerization data calculated from infinite dilution values of the viscosity function and using the Kraemer relationship (7), are compared with degree of polymerization data calculated on the basis of the value of the viscosity function at 0.5% concentration.

Relationship between Apparent and Basic Degree of Polymerization. It is routine practice in many laboratories to determine the viscosity (or fluidity) of a solution of cellulose in cuprammonium solvent at a standard concentration high enough to make the viscosity measurement as simple as possible. In this way, it is practical to determine relative changes in viscosity and thereby obtain a measure of the degree of depolymerization of cellulose. The arbitrary standard concentrations most widely used are 0.50 and 1.0%, respectively.

The data presented in this paper correlate the values of the viscosity function obtained at the standard concentration of 0.50% (apparent D.P.) for five representative samples of cellulose with the values for the respective viscosity functions obtained at infinite dilution (basic D.P.):

$$\text{Limit}\left(\frac{\eta_{sp}}{c}\right) c \to 0$$

On plotting the $\frac{\eta sp}{c}$ *vs.* c data for each sample on ordinary graph paper, the curves in Figure 1 are obtained, in which the rate of increase of the slope is dependent upon the degree of polymerization of the sample being studied. Furthermore, as the degree of polymerization increases, the variation in the slope of the curves even at very low concentrations precludes reliable extrapolation of the $\frac{\eta sp}{c}$ *vs.* c data. However, when the same data are plotted on semilogarithmic paper, linear curves are obtained for each sample (Figure 2) and more reliable extrapolation is possible.

The data have been used to draw a conversion graph (Figure 3) in which the calculated "apparent D.P." obtained for each sample at 0.5% concentration are plotted against the corresponding "basic D.P." calculated from the values of the respective viscosity functions at infinite dilution, using the Kraemer relationship (7).

A more applicable conversion relationship has been obtained by plotting the values of $(\eta r + 1)$, determined from the solution viscosity at 0.5% concentration, against the corresponding values for the "basic D.P." on semilogarithmic graph paper. When this is done, a linear relationship is obtained and is expressed by:

$$\text{Basic D.P.} = a[\log (\eta r + 1) - b] \quad (1)$$

where a and b are constants representing the slope and intercept, respectively, of Figure 4.

The values of constants a and b were obtained graphically from Figure 4, and on substituting them in Equation 1 we obtain:

$$\text{Basic D.P.} = 2160\ [\log (\eta r + 1) - 0.267] \quad (2)$$

The Kraemer relationship and constant (7) for cellulose in cuprammonium solution used to calculate degree of polymerization data from values of the viscosity function at infinite dilution are given in Equation 3:

$$\text{D.P.} = 260\ [\eta] \quad (3)$$

where $[\eta]$ is the value for the intrinsic viscosity at infinite dilution

$$\text{Limit } \left(\frac{\ln \eta r}{c}\right) c \to 0$$

Table II. Comparison of Degree of Polymerization Data Obtained by Two Methods of Calculation

Material	Degree of Polymerization: From infinite dilution data using Equation 3	Degree of Polymerization: From data at 0.5% concentration using Equation 2
Raw cotton	3120	3100
Absorbent cotton	1950	1980
Normal viscosity wood pulp	960	965
Low viscosity wood pulp	750	735
Viscose rayon	390	408

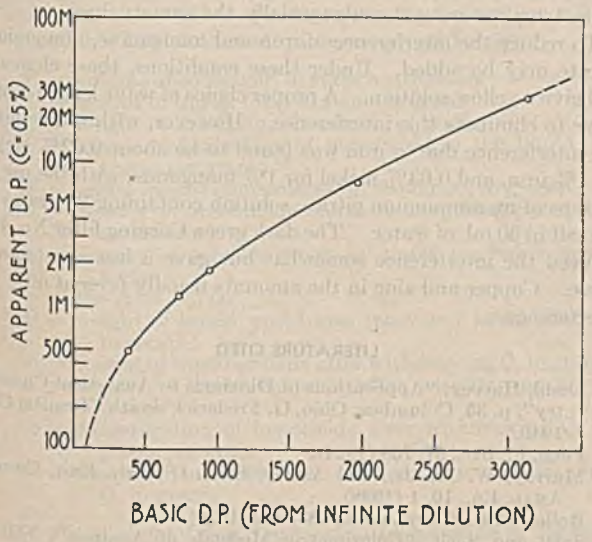

Figure 3. Conversion Graph

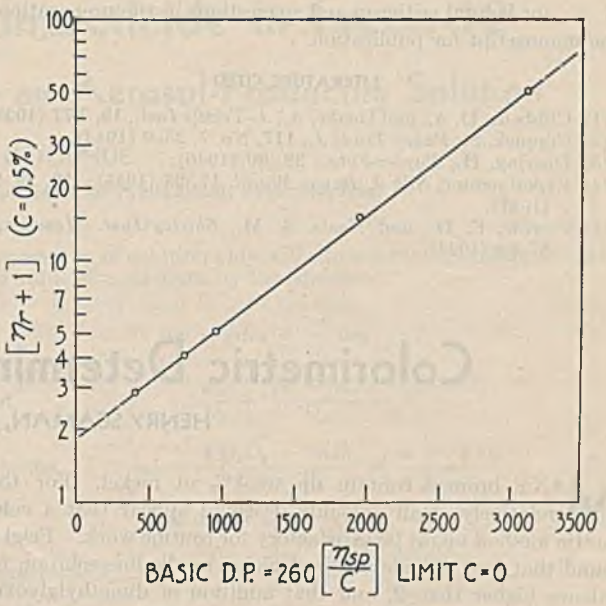

Figure 4. Conversion Relationship

CONCLUSIONS

When viscosity-concentration data, obtained for five representative samples of purified cellulose covering the degree of polymerization range from 300 to 3000, are plotted on semilogarithmic paper, linear relationships are obtained in each case. This permits more accurate extrapolation of the viscosity data to obtain the intercept values of the viscosity function—i.e., values at infinite dilution—from which degree of polymerization data may be calculated.

The logarithmic relationship between the values for the viscosity function $(\eta r + 1)$, obtained at 0.5% concentration, and the respective degree of polymerization data calculated from the values of viscosity function at infinite dilution, is also linear and is expressed by Equation 2. The numerical constants of this equation were obtained by a graphical analysis of the data.

A conversion graph has been drawn relating the "apparent" degree of polymerization obtained at 0.5% concentration to the corresponding values for the degree of polymerization obtained by extrapolation to infinite dilution (Figure 3).

Equation 2 may be used for accurately converting values of the viscosity function obtained at the standard concentration of 0.50% to basic degree of polymerization data equivalent to values obtained by extrapolation of viscosity-concentration data to infinite dilution and using the Kraemer relationship and constant (7).

ACKNOWLEDGMENT

The writer is indebted to S. Coppick, acting professor of forest chemistry, The New York State College of Forestry, Syracuse,

N. Y., for helpful criticism and suggestions in the preparation of the manuscript for publication.

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Colorimetric Determination of Nickel in Bronze

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MANY bronzes contain up to 1% of nickel. For these relatively small amounts it would appear that a colorimetric method might be satisfactory for routine work. Feigl (*2*) found that lead dioxide oxidized nickel in alkaline solution to a valence higher than 2, and that addition of dimethylglyoxime to this solution gave a red coloration rather than a precipitate. This procedure was improved by Rollet (*4*), who used bromine water instead of lead dioxide, and this method has found many applications (*1*, *3*, *5*). This reaction has been applied to the determination of nickel in bronze using a filter photometer such as the Cenco photelometer with a cell 10 mm. thick, taking about 17 ml. of solution.

PROCEDURE

After the tin is removed by filtration as metastannic acid and the copper and lead by electrolysis, the remaining solution is diluted to 150 ml. and mixed. One milliliter is transferred by pipet to a 100-ml. tall-form beaker, 25 ml. of distilled water are added, and the mixture is shaken after addition of one drop of saturated bromine water. Seven drops of an ammoniacal solution of dimethylglyoxime (10 grams of dimethylglyoxime dissolved in 650 ml. of ammonium hydroxide and diluted to 1 liter) are added and the mixture is again shaken well. The orange-red color develops in alkaline solution immediately on shaking. The solution is transferred to a photelometer cell and the absorption determined with the use of the Cenco dark blue filter or a Corning blue filter such as No. 556. The maximum absorption occurs at 475 mμ. The per cent nickel is obtained from the usual type of straight-line curve plotted on semilog paper. The calibration data for this curve can be obtained through the use of a solution of a C.P. nickel salt standardized gravimetrically, or preferably by removing an aliquot from the regular sample, obtaining the colorimetric value from this aliquot, and using the remainder for a gravimetric determination. With bronzes containing manganese, iron, or aluminum, 3 to 5 drops of a solution of ammonium citrate (25 grams of ammonium citrate dissolved in 30 ml. of water) are added before addition of bromine water.

RESULTS AND DISCUSSION

Some typical single results obtained by this method are shown in Table I. In general, it is believed the results are satisfactory for the usual type of bronze. The use of ammonium citrate does not eliminate the interference of manganese and iron but reduces it considerably. The precision and accuracy of this method in the range indicated are 0.02 to 0.04% nickel.

To obtain satisfactory results with this method it is necessary to standardize on a procedure and use it for all determinations. Among the factors which can affect the intensity of the color are time of standing, amount of bromine used, amount of ammonium citrate used, shaking, and temperature. The color intensity increases on standing, the increase being greatest during the first 20 minutes, and tends to level off after 2 hours. A typical increase during the first 20 minutes would be from 0.58 to 0.60% nickel. The use of more than one drop of bromine water and the use of ammonium citrate tend to lower the color intensity slightly—for example, standard 52a gave 0.75% nickel with 1 drop of bromine water, 0.74% with 2 drops, and 0.71% with 5 drops. An approximately equal reduction in values for nickel is obtained with 3 to 5 drops of the ammonium citrate solution, so that 5 drops of bromine water and 5 drops of the ammonium citrate solution give values of 0.67 to 0.68% nickel for this sample. Low results will also be obtained by the use of too small a drop of bromine water, in which case some nickel will be precipitated.

Table I. Nickel Determinations on Bureau of Standards Samples

Sample No.	Interfering Elements	Nickel Gravimetric	Nickel Colorimetric
	%	%	%
37C	Fe, 0.17	0.58	0.59
37C	Fe, 0.17	0.58	0.57[a]
37B	Fe, 0.21	0.45	0.46[a]
37B	Fe, 0.21	0.45	0.46[a]
52	Fe, 0.12	0.13	0.13
52	Fe, 0.12	0.13	0.13[a]
124	Fe, 0.38	0.45	0.47
124	Fe, 0.38	0.45	0.46[a]
52a	Fe, 0.05; Mn, 0.02	0.73	0.75
62	Fe, 1.13; Mn, 1.59; Al, 1.13	0.64	0.70[a]
Manganese bronze[c]	Fe, 2.2; Mn, 3.1; Al, 3.8	0.00	0.10[b]

[a] Using 3 drops of ammonium citrate solution, 25 grams per 30 ml.
[b] Using 5 drops of ammonium citrate solution, 25 grams per 30 ml.
[c] A commercial sample.

Some experiments indicate that fairly vigorous shaking is necessary to develop the maximum color intensity, although the values obtained were indecisive. Temperature has little effect on the color, except that a hot solution will give a precipitate rather than a color. Temperatures somewhat above or below room temperature gave substantially the same values.

To reduce the interference of iron and manganese, ammonium citrate may be added. Under these conditions, these elements will give a yellow solution. A proper choice of wave length might serve to eliminate this interference. However, with a blue filter the interference due to iron was found to be about 0.02% nickel for 1% iron, and 0.03% nickel for 1% manganese with the use of 3 drops of an ammonium citrate solution containing 25 grams of the salt in 30 ml. of water. The dark green Corning filter No. 401 reduced the interference somewhat but gave a less satisfactory curve. Copper and zinc in the amounts usually present offer no interference.

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Increase in Concentration of Insecticide in Freon-12

Accompanying Transfer or Discharge of an Aerosol-Producing Solution

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In the transfer or discharge of solutions in liquefied gases used for the production of insecticidal aerosols, a concentrating effect occurs because of escape of solvent from the solution to maintain the high vapor density. A mathematical treatment of this effect is given, and experiments are described by which it was confirmed for solutions in Freon-12. In that case a discharge of 90% of the liquid phase raises the concentration of the remaining solution by 8% of its value.

THE insecticidal aerosol, produced when a solution of insecticides in a liquefied gas is released into the air (*3*), has met an urgent military need, especially for disinfesting airplanes and for overseas use. The combination of pyrethrum and sesame oil in dichlorodifluoromethane (Freon-12) produces a very effective nonflammable insecticide that is nontoxic to man and animals. In the manufacture and packaging of this solution, certain questions have arisen concerning the physical properties of solutions in liquefied gases. A mathematical treatment of one of these problems, which has to do with change in concentration due to transfer or discharge of insecticide, and confirmatory experimental results are presented in this paper.

At 80° F. (26.7° C.) the density of saturated Freon-12 vapor is 0.0377 gram per cc. As liquid is withdrawn from an aerosol container during use, appreciable quantities of Freon evaporate from the solution remaining in the container to maintain this high vapor concentration in the increasing space not occupied by the liquid. As a result the concentration of the remaining solution gradually increases as the container empties. While this change is less serious than if the solution progressively weakened, still the need for conservation of insecticide suggests that some consideration be given to the matter, especially in connection with packaging the solution.

MATHEMATICAL DEVELOPMENT

An approximate estimate of the magnitude of this effect at any given fixed temperature can be obtained by means of the calculus, if it is assumed that the densities of both gaseous and liquid phases remain constant. This is obviously not strictly true, but it will be shown later that the departure from exactness is inconsequential. The mathematical development follows:

Let V = volume of container, in cubic centimeters
M = weight of initial total content, in grams
M_s = weight of initial liquid content, in grams
C = initial concentration of insecticide in the liquid, in grams per gram
W = initial weight of insecticide in the liquid, in grams
D_s = density of insecticide solution, in grams per cubic centimeter
D_g = density of solvent vapor, in grams per cubic centimeter
r = the ratio D_g/D_s
Q = weight of liquid withdrawn (no vapor being allowed to escape)
m = weight of total contents after withdrawing Q, in grams
m_s = weight of liquid contents after withdrawing Q, in grams
c = concentration of insecticide after withdrawing Q, in grams per gram
w = weight of insecticide in container after withdrawing Q, in grams

At any stage of emptying, the weight of insecticide in the container is w. Withdrawal of an additional infinitesimal weight, dm, consisting of solution only will cause a corresponding change in the value of w, as given by the equation

$$dw = c\,dm = \frac{w}{m_s}\,dm \qquad (1)$$

But $$\frac{m_s}{D_s} + \frac{m - m_s}{D_g} = V$$

Whence $$m_s = \frac{VD_sD_g - mD_s}{D_g - D_s} = \frac{m - VD_g}{1 - r} \qquad (2)$$

and $$dm = (1 - r)\,dm_s \qquad (3)$$

From 1 and 3 $$\frac{dw}{w} = \frac{(1 - r)\,dm_s}{m_s}$$

Proceeding to definite integrals, $$\ln \frac{w}{W} = \ln \left(\frac{m_s}{M_s}\right)^{1-r}$$

Therefore, since $w = m_s c$ and $W = M_s C$

$$\ln \frac{m_s c}{M_s C} = \ln \left(\frac{m_s}{M_s}\right)^{1-r}$$

from which $$\frac{c}{C} = \frac{M_s}{m_s} \times \frac{m_s^{1-r}}{M_s^{1-r}} = \left(\frac{m_s}{M_s}\right)^{-r}$$

If M_s is known, as it was in some of these laboratory experiments because of the manner of filling, m_s at any stage is calculable from it and the weight, Q, of liquid withdrawn, for from Equation 2

$$m_s = \frac{M - Q - VD_g}{1 - r} = M_s - \frac{Q}{1 - r}$$

and hence $$\frac{c}{C} = \left[1 - \frac{Q}{(1 - r)M_s}\right]^{-r} \qquad (4)$$

By the aid of values calculated from this equation, the percentage increase in concentration, $100\,\frac{c - C}{C}$, can be plotted against $\frac{100\,Q}{M_s}$, the percentage of solution withdrawn, as is illustrated in Figure 1 for a solution containing 5% of sesame oil in Freon-12. For the construction of this graph D_g was taken as 0.0377 gram per cc., D_s as 1.291 grams per cc., and r therefore as 0.0292. It shows that a quantity of liquid equal to 22% of the original liquid content must be withdrawn before the concentration of insecticide rises 1%, that removal of 80% causes a 5% rise, and that 92.5% delivery gives only a 9% rise.

If, as will more often be the case, the total content of the container, M, rather than the liquid content, M_s, is known, Equation 4 can be converted, because of the relationship

$$M_s = \frac{M - VD_g}{1 - r}$$

into the equivalent form

$$\frac{c}{C} = \left(1 - \frac{Q}{M - VD_g}\right)^{-r}$$

and $100\,\frac{c - C}{C}$ can be plotted against $\frac{100\,Q}{M}$, the percentage of total contents removed.

EXPERIMENTAL VERIFICATION

The errors due to the assumption that the gas and liquid densities are constant can be judged by a consideration of the possible departures of those values from constancy. The possible changes in gas density were derived from measurements of the lowering

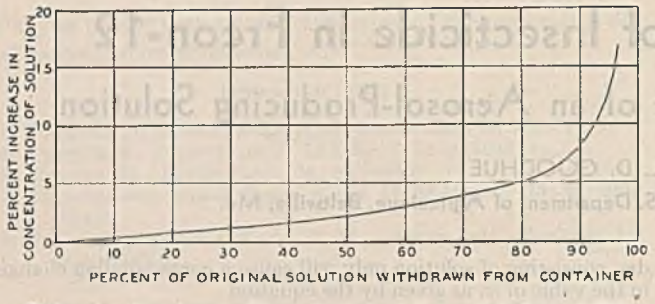

Figure 1. Increase in Concentration of Freon Solution of Oily Insecticide as Contents Are Withdrawn from Container

Table I. Lowering of Vapor Pressure of Freon-12 by Sesame Oil

Sesame Oil	Vapor Pressure Lowering
%	*Mm. of Hg*
2.5	6, 7, av. 6.5
5.0	18, 19, 18, av. 18.3
10	23, 24, 28, av. 25
15	35, 37, av. 36

of vapor pressure produced by dissolving various proportions of sesame oil in Freon-12.

The apparatus used to make these measurements (Figure 2) consists of two identical containers with valves connected through a U-tube containing mercury, which acts as a differential manometer. Two small petcocks, one on each side of the manometer, are necessary to operate the apparatus.

Three hundred grams of liquid Freon-12 were always placed in the container on the right and an equal weight of solution was made up in the one on the left. The connections were made to the manometer, and the vapors from the two containers were allowed to enter the manometer simultaneously until the total vapor pressure on each side was exerted. The whole apparatus was then submerged in a transparent water bath. Readings were made at 80° F. after the system had reached equilibrium.

The Freon-12 used contained some nonliquefiable gases, which interfered somewhat. To overcome this interference, containers of the same size with the same volumes occupied by the liquid on

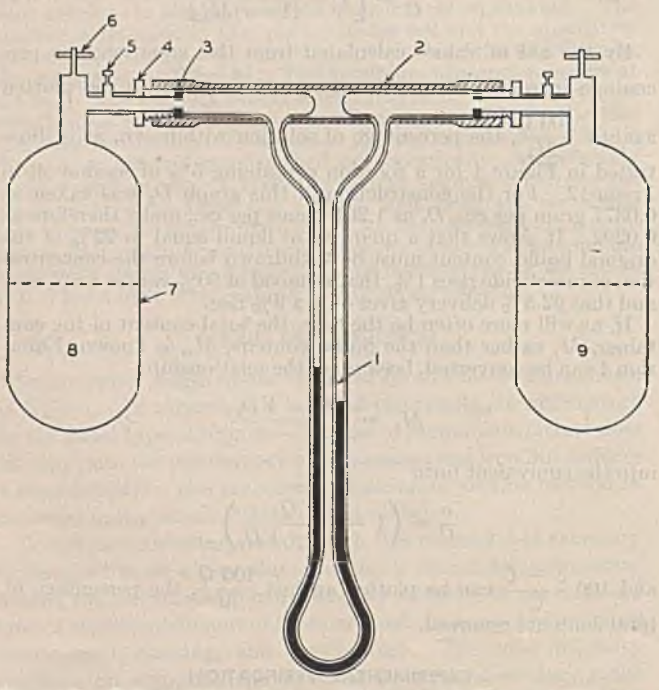

Figure 2. Differential Manometer Apparatus to Determine Vapor Pressure Lowering of Liquefied Gas Solutions

1. Heavy-walled glass tube containing mercury
2. Brass frame from 0.5-inch pipe having slots cut in each side
3. Rubber gasket
4. Adapter from 0.375- to 0.125-inch pipe thread
5. Petcock
6. Needle valve on container
7. Container
8. Liquefied gas solution
9. Liquefied gas

both sides were used. The exact amount of Freon needed for the solution was introduced to avoid fractionation by the removal of any excess. The results are shown in Table I.

Since the total vapor pressure of Freon-12 is about 5000 mm. of mercury, the degree of reproducibility shown is considered very good.

Fifteen per cent of nonvolatile material has been found to be about the optimum that should be used in an aerosol solution. Such a solution will have a vapor pressure about 36 mm. of mercury below that of Freon-12. Reference to the equation of state derived for Freon-12 by Buffington and Gilkey (*2*) shows that this lowering of pressure produces a change in vapor density of only 0.0003 gram per cc., which for the authors' purposes can be considered negligible in comparison with the figure 0.0377 gram per cc. used in constructing the graph.

The possible changes in liquid density were evaluated by consideration of the values for density of solutions of sesame oil in Freon-12, determined at 80° F. by means of a small hydrometer in a closed system.

The liquid was placed in a pressure test tube together with the hydrometer. The whole apparatus (Figure 3) was set in a glass water bath at 80° F. and the length of the emergent stem was determined with a cathetometer. The hydrometer was calibrated by observing the length of the emergent stem above the Freon at several temperatures. The error of this calibration due to the increasing vapor density above the Freon was calculated and found to be negligible. A curve was plotted from which the densities of the various sesame oil solutions were determined at 80° F. (26.7° C.). The values obtained are shown in Table II.

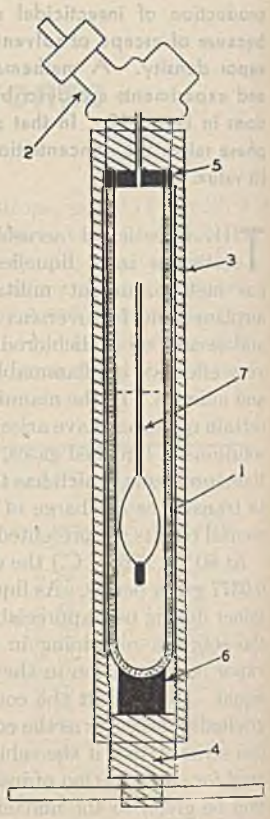

Figure 3. Pressure Test Tube Assembly and Small Hydrometer

1. Heavy-walled glass test tube 10 mm. in inside diameter and 155 mm. long
2. Standard Y-valve for small refrigerant drums
3. Frame from 0.5-inch brass pipe with long windows cut in opposite sides
4. Screw plug
5. Gasket
6. Rubber cushion
7. Hydrometer

Since 15% of oil is taken as the optimum, the change in liquid density will not exceed 0.048 gram per cc., which would produce a change of only about 0.3 in the value of $100\,\frac{c - C}{C}$ calculated for the case in which 90% of the contents of the tank is withdrawn. Thus it appears that the graph is sufficiently accurate for all ordinary purposes.

As an objective confirmatory test, measurements of the increase in concentration were made on 400-gram samples of an approximately 5% solution of cottonseed oil in Freon-12. Cotton-

Table II. Change in Density of Freon-12 Due to Sesame Oil

Sesame Oil	Density
%	*G./cc.*
0	1.303[a]
2.5	1.298, 1.2985, av. 1.298
5.0	1.290, 1.292, av. 1.291
10	1.274, 1.274, av. 1.274
15	1.2548, 1.2552, av. 1.255
20	1.2285, 1.2295, av. 1.229

[a] From Bichowsky and Gilkey (*1*).

Table III. Concentrating Effect Caused by Removal of Liquid from an Aerosol Bomb

(Containing originally 400 grams of a 5% solution of cottonseed oil in Freon-12)

Solution Withdrawn % by weight	Relative Concentration of Oil Determined	Calculated
	%	%
0	(100.0)	100.0
50	100.9, 101.5, av. 101.2	102.1
80	105.3, 106.5, av. 105.9	105.2
90	108.9, 109.1, av. 109.0	108.0

seed oil was chosen instead of sesame oil because it did not oxidize when heated for the analyses. The initial concentration was determined by withdrawing two 5-gram samples into pressure test tubes, which were weighed before and after to determine the exact weight of the samples.

The apparatus was used without the hydrometer. The volatile solvent was then allowed to evaporate, and the test tube containing the residue was removed from the frame and heated for 30 minutes at 110° C. The weight of the residue was then determined and the concentration by weight of nonvolatile matter calculated. Duplicate samples were also taken after 50, 80, and 90% of the solution had been allowed to escape. Mechanical difficulties made the results unreliable after 95% had been removed. All operations were carried out at 80° F. The results are shown in Table III.

The degree of concordance shown is good, considering the experimental difficulties involved. The over-all effect is comparatively small until the container is almost empty and, since it is in the direction leading to greater assurance of getting the required minimum concentration, not very important in the actual application of the insecticide. It might be important, however, to a manufacturer filling small containers from a large one. The last containers to be filled will contain more insecticide than the first unless some compensatory measures are taken. It is also important when samples of solution are being used for test purposes as a standard of comparison. In precise laboratory tests it would be good practice to use not more than 50% of the original solution. The simplest procedure in the commercial filling of aerosol containers is to add sufficient pure Freon to the reservoir at intervals to maintain approximately the original concentration. This procedure is used by some present aerosol manufacturers.

Although the foregoing discussion has been based wholly on data pertaining to dichlorodifluoromethane, the formula developed will obviously apply to all liquefied-gas solutions for which the densities of the liquid and gaseous phases remain reasonably constant during evaporation.

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A Modified Bailey Buret

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THE San Antonio Army Service Forces Depot Laboratory has, for some time, been analyzing large numbers of samples of mayonnaise and other semisolid salad dressings. Tests have been made according to the methods of the Association of Official Agricultural Chemists (*1*).

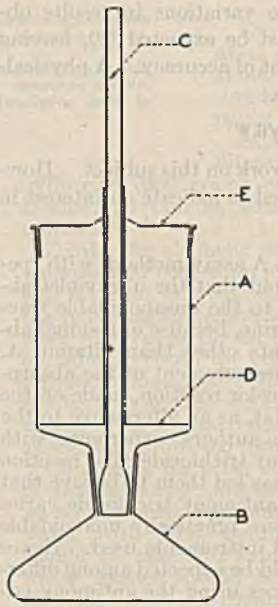
Weighing out such samples with the Bailey weighing buret (*2*) has never proved satisfactory. The only size available to the authors has been the 30-ml. capacity which holds an insufficient quantity of salad dressing if duplicate determinations are to be made. Salad dressings, moreover, are of such consistency that there is almost no flow of material from the tip of the buret due to gravity alone. Forcing the sample out with the plunger is a very slow process and results in the accumulation of a considerable quantity of material on the adapter and on that portion of the plug which projects above. Such a situation inevitably results in loss.

A fairly simple modification of the Bailey buret was decided upon as the best solution of this problem. The changes involved were enlarging the buret to a capacity of 100 ml., straightening out the top of the buret completely, eliminating the constriction, and adding a plunger to go down inside the buret and around the plug. All clearances were kept to a minimum.

Buret *A* is constructed from 51-mm. Pyrex glass tubing with outside 50/12 ⅀ joint at top and inside 15/20 and outside 10/18 ⅀ joint at the constricted bottom. Distance between upper joint and beginning of constriction is 50 mm. Flask *B* has outside 15/20 ⅀ joint and over-all diameter of 67 mm. Plug *C* is a 6-mm. glass rod, 165 mm. in length (over-all), and contains inside 10/18 ⅀ joint.

Plunger *D* is of 18-8 8-mm. ($^1/_{32}$-inch) stainless steel, consisting of a tube and disk spot-welded together. Inside diameter of tube and diameter of disk are of size to give snug fitting around plug and inside buret, respectively. Adapter or stopper *E* is hollow-ground and has inside 50/12 joint and orifice to fit over tube *D*.

The modified buret is filled while sitting on the flask base *B*, with plug *C* in place. Plunger *D* is then fitted around the plug



and allowed to rest on the material The adapter, *E*, is finally placed in position, and the assembly is weighed. To remove the sample, the plug is raised and held in open position and, with the same hand, the plunger is pressed downward, forcing out the material. When enough sample has been taken, the plug is pushed into the joint, pressing out the last drop before the buret is returned to the flask.

It is immediately apparent that the total weight of the assembly, filled, is too great for the capacity of an ordinary analytical balance. The assumption is that the larger samples are to be weighed on a more rugged balance, an accuracy of 0.1 or at most 0.01 gram being adequate.

The modified Bailey buret should find use in analyzing samples, such as soft grease, paste paints, certain asphalts, water-repellent emulsions, and other semisolid materials. The plunger, too, can always be removed and the buret can be used to an advantage for any bulky sample.

Satisfactory working models of this buret were obtained from the Scientific Glass Company, Bloomfield, N. J., and have been in continuous use with marked success during the past year. Steps are now being taken to remove minor defects in the design.

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Determination of Vitamin A Content of Margarine

Spectrophotometric Method

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A spectrophotometric method for the determination of vitamin A in commercial margarine has been developed. It is based on the destruction of vitamin A in a portion of a solution of the unsaponifiable fraction of margarine fat by ultraviolet light irradiation, and the use of this devitaminized solution as a control for the spectrophotometric determination of vitamin A in a second portion of the original unsaponifiable solution not irradiated with ultraviolet light. The validity of the method is established by a comparison of the results obtained with results of biological assays made on identical samples.

THE need for a rapid and accurate method for the determination of the vitamin A content of margarine has existed as long as margarine has been fortified with vitamin A, but has become more pressing during the last few years as a result of the present governmental nutritional program which encourages the enrichment and fortification of certain foods with essential nutrients. In 1941, a federal definition and standard of identity for oleomargarine (*7*) was promulgated, which requires that margarine vitaminized with vitamin A must carry not less than 9000 U.S.P. units of vitamin A per pound in the finished margarine. Since this standard has been in effect, almost all margarines on the market carry vitamin A; hence, a dependable and rapid method for determination of vitamin A in finished margarine has recently become relatively more important.

The biological method is too time-consuming for production control purposes. In addition, wide variations in results obtained by the biological method must be expected (*2*), leaving much to be desired from the standpoint of accuracy. A physical-chemical method is therefore desirable.

LITERATURE REVIEW

There is relatively little published work on this subject. However, sufficient work has been published to indicate an interest in this matter.

Edisbury (*6*), in describing vitamin A assay methods with special reference to margarine, pointed out that the ultraviolet absorption method, even when applied to the unsaponifiable fraction of the oil, is unreliable for margarine, because of residual absorption of unsaponifiable constituents other than vitamin A. He suggested a spectrophotometric measurement of the absorption due to the antimony trichloride color reaction, made on the unsaponifiable fraction of margarine fat, as an alternative to the biological assay of margarine. The authors' experience with measuring the intensity of the antimony trichloride color reaction as a means of estimating vitamin A has led them to believe that the intensity of color produced with antimony trichloride varies considerably with slight and oftentimes practically unavoidable variations in details of technique and instruments used. Hence considerable variations in results would be expected among different operators and different laboratories using the antimony trichloride method of vitamin A determination for control purposes. In addition, certain ingredients used in some margarines tend to alter the color produced by the action of antimony trichloride on vitamin A.

Oser (*10*) indicates that an adaptation of the Dann and Evelyn (*3*) antimony trichloride method is useful for a quantitative control of vitamin A in margarine. The improvements in this method consist of a correction for side reactions which sometimes develop interfering color or turbidity, and the inclusion of a density measurement produced by the addition of a known increment of vitamin A. The Evelyn photoelectric colorimeter is claimed to increase the accuracy of reading the blue color developed at its maximum intensity. However, this method still depends on being able to read, at its maximum intensity, the unstable and somewhat fleeting color developed by the reaction of antimony trichloride with vitamin A. The instability of the color developed is troublesome for control purposes.

This laboratory (*9*) has published a spectrophotometric method for determination of vitamin A in dairy butter, based on: (*a*) the fact that vitamin A is characterized by a maximum absorption at the 3280 Å. band and can be measured by determining the intensity of absorption in the ultraviolet region at this (3280 Å.) wave length, as pointed out by Morton *et al.* (*5*, *8*) and (*b*) the fact that vitamin A is destroyed by ultraviolet light; within recent years, Demarest (*4*) has published the results of his studies on the destructive irradiation of vitamin A. Thus by destroying the vitamin A (and carotene) in a portion of the unsaponifiable fraction, a control is obtained for the spectrophotometric determination of the vitamin A and carotene contained in the sample. This method is considered to yield satisfactory results.

EXPERIMENTAL

Attempts were made to apply this spectrophotometric method (*9*) to margarine, following the procedure used for dairy butter; however, certain modifications are necessary. The difficulties apparently are due primarily to the fact that the unsaponifiable content of domestic U. S. vegetable oils, from which most present-day U. S. margarines are made, differs considerably, both in composition and amount, from the unsaponifiable content of butterfat. Another factor concerned is the fact that butterfat may be more readily saponified than domestic vegetable oils.

Preliminary experimental work indicated that most progress could be made by modifying the method as applied to dairy butter (*9*) in the following respects:

1. Increasing saponification time in order to ensure complete saponification of the margarine fat before extraction of the unsaponifiable matter.
2. Increasing the number of extractions and the quantity of solvent used for extraction of the unsaponifiable, in order to ensure complete extraction of the unsaponifiable material containing vitamin A.
3. Increasing the time of ultraviolet light irradiation of the unsaponifiable in solvent solution, in order to overcome the masking effect of the relatively large amount of unsaponifiable material present in margarine fat and thus to obtain complete destruction of the vitamin A contained therein.

For use in this determination, all solvents must be exceedingly pure—a point which cannot be overemphasized. The solvent for the unsaponifiable material extracted from the oil must be optically clear, must possess adequate solvent power to hold in solution at normal room temperatures the amount of unsaponifiable present in the sample, must be of sufficiently high boiling point to permit long exposures under an ultraviolet lamp which generates considerable heat, and must have no destructive effects upon the vitamin A dissolved therein, at least for several hours.

The authors have found either cyclohexane or methyl cyclohexane, specified as "purified for spectrophotometric use and free of extraneous ultraviolet absorption" and obtained from Eastman Kodak Company, Rochester, N. Y., to be satisfactory in most instances (*9*). Vitamin A is stable in either of these solvents for several days, provided that the solutions are stored in the dark, and the solvents themselves are sufficiently pure.

It has been the authors' practice to verify the suitability of each lot of either cyclohexane or methyl cyclohexane by spectrophotometric comparison with a sample of known purity. A sample is considered usable only if it shows no extraneous absorption in the region between 5000 and 2200 Å.; extraneous absorption within this range is considered evidence of impurities and the material is rejected. A method satisfactory for the purification of cyclohexane containing a small amount of impurities has been given (*9*), but is not satisfactory for the purification of methyl cyclohexane.

The ether and alcohol used for extracting the unsaponifiable material must be especially pure and free of peroxides, in order to

avoid oxidation of the extracted vitamin A. These solvents must be carefully purified before use, even though the best grades are purchased. A method for the purification of anhydrous C.P. ethyl ether was given in a previous publication (*9*). Specially denatured No. 30 alcohol can be satisfactorily purified by an A.O.A.C. method (*1*).

Repeated attempts have demonstrated that the vitamin A content of whole margarine fat (vitaminized) cannot successfully be destroyed by ultraviolet light irradiation, using available ultraviolet light equipment. However, the vitamin A content of the unsaponifiable fraction of margarine fat can be destroyed by intense and ultraviolet irradiation. A considerably more transparent solution of the extracted unsaponifiable, in the range below about 3200 Å., results from the intense ultraviolet irradiation required to destroy completely the vitamin A in the unsaponifiable fraction of margarine fat; at 3280 Å., the increased transparency due to the action of ultraviolet light on unsaponifiable materials other than vitamin A is so small as to be neglected for all practical purposes. This point is demonstrated by the results shown in Table I.

The points covered in Table I are further illustrated in Figure 1, in which are shown ultraviolet absorption curves obtained on samples not included in the table. These curves, all obtained from the unsaponifiable fractions of the same stock of margarine oil, serve to emphasize:

The absorption due to constituents other than vitamin A in margarine oil unsaponifiable.

Ultraviolet irradiation of the unsaponifiable fraction of a vitaminized oil produces a material which shows no vitamin A characteristics spectrophotometrically. This irradiated material shows an absorption, at the point of maximum vitamin A absorption, in reasonably close agreement with that of the nonvitaminized, nonirradiated, unsaponifiable fraction of the same oil.

Irradiation increases the transparency of the unsaponifiable fraction in regions below about 3200 Å.

Irradiation of the unsaponifiable fraction of a vitaminized margarine oil produces a control for spectrophotometric vitamin A determinations, which gives results in reasonably close agreement with those obtained by use of the unsaponifiable fraction of the same oil before vitaminizing.

The curve (curve 5) obtained by the method described herein exhibits a vitamin A peak at the region of maximum vitamin A absorption.

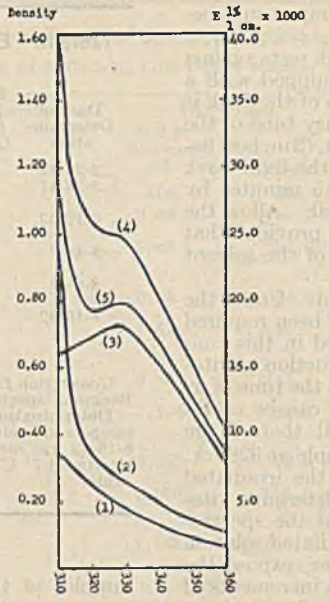

Figure 1. Ultraviolet Absorption Curves

(1) Irradiated unsaponifiable fraction of vitaminized margarine oil *vs.* solvent control
(2) Nonirradiated unsaponifiable fraction of same margarine oil before vitaminizing *vs.* solvent control
(3) Nonirradiated unsaponifiable fraction of same vitaminized margarine oil *vs.* nonirradiated unsaponifiable fraction of same oil before vitaminizing (both in solvent)
(4) Nonirradiated unsaponifiable fraction of same vitaminized margarine oil *vs.* solvent control
(5) Nonirradiated unsaponifiable fraction of same vitaminized margarine oil *vs.* same material after irradiation (both in solvent)

The following method is based on the destruction of vitamin A in a portion of a cyclohexane (or methyl cyclohexane) solution of the unsaponifiable fraction of margarine fat by ultraviolet light and the use of this devitaminized solution as a control for the spectrophotometric determination of vitamin A in a second portion of the original unsaponifiable solution not irradiated with ultraviolet light.

EQUIPMENT USED

Spectrophotometer. Adam Hilger, Ltd., intermediate quartz spectrograph with Spekker photometer, equipped with tungsten steel electrodes as a source of light. Quartz absorption cells, Hilger Type C, 1-cm. quartz Kjeldahl-shaped flasks, 25 cc.

Beckman quartz spectrophotometer, Model D, manufactured by National Technical Laboratories, South Pasadena, Calif. Equipped with tungsten lamp light source, cesium oxide and blue sensitive phototubes, and Corex absorption cells (1-cm. square type).

Both instruments have been used with equally satisfactory results for this determination.

Ultraviolet Lamp. Uviarc poultry treater, Type RT, Spec. 100, Cooper-Hewitt Electric Co., Hoboken, N. J. A more complete description of this lamp, together with a reference for obtaining its spectral radiation, has been given (*9*).

METHOD

Extraction of Unsaponifiable Material. Melt the margarine in a water bath at about 60° C., and separate the fat by filtration through a Whatman No. 12 folded filter paper (or other equivalent paper).

Saponify 20 grams of the separated and filtered fat with 30 cc. of alcoholic potassium hydroxide (200 grams per liter of specially denatured No. 30 alcohol) by boiling, with suitable reflux arrangement, for 15 minutes.

Dilute the alcoholic solution with water to approximately 4 volumes and cool in an ice-water bath. Extract the unsaponifiable material with cold ethyl ether. At least six extractions, with the following successive amounts of ether, are required for complete removal of the unsaponifiable material: 200, 150, 100, 50, 50, 50 cc. (Both the ether and alcohol must be very carefully purified immediately before use. This is essential for dependable results.) Vigorous shaking is also necessary for complete removal of the unsaponifiable material. The sample should be adequately protected from sunlight during extraction.

Composite the ether extracts and wash with 150-cc. portions of distilled water until substantially free of soap. (There should be no appreciable turbidity developed after acidifying the wash water with 10% hydrochloric acid.) Adequate precautions should be taken to avoid troublesome emulsions; the first two water washes should be made by merely pouring the water through the ether without shaking.

Filter the ether solution through filter paper and concentrate to 25 to 50 cc. by distillation on a steam bath. Remove the remainder of the ether by evaporating, on a steam bath, under a stream of carbon dioxide to prevent oxidation. When substantially all the ether has been evaporated, cool immediately to about 20° C. (70° F.) and dissolve the unsaponifiable material in optically clear cyclohexane or methyl cyclohexane. Make a solution up to 50 cc. (40% solution weight to volume, on original fat basis). Filter and store in the dark at about 4° to 10° C. (40° to 50° F.) until the sample is examined spectrophotometrically, but not longer than 48 hours.

Spectrophotometric Determination of Vitamin A. Divide the cyclohexane (or methyl cyclohexane) solution of unsaponifiable material into two parts, and irradiate one portion under the Uviarc as follows: Transfer the solution to be irradiated into a 25-cc. Kjeldahl-shaped quartz flask, and stopper the flask with a cork wrapped in aluminum foil. Allow at least 10

Table I. Effect of Irradiation

(Comparison of controls used in the unsaponifiable method for determination of vitamin A in margarine oil. Duplicate determinations)

Sample No.	Date Analyzed	Irradiated Unsaponifiable Control	Nonirradiated Unsaponifiable of Oil before Vitaminizing as Control	Irradiated Unsaponifiable of Vitaminized Margarine Oil *vs.* Nonirradiated Unsaponifiable of Same Oil before Vitaminizing
		U.S.P. units of vitamin A per pound of margarine		
1	6-11-42	13,600	14,000	600
		13,600	14,000	600
2	6-17-42	15,000	14,200	600
		16,100	15,100	600

Samples prepared under authors' supervision, with process samples available for use in this experimental work.

minutes for the lamp to come to full operating temperature before starting irradiations.

Support the flask in such a position that the cork rests against the rim of the lampshade (the lamp in use is equipped with a shade 21 cm., 14 inches, in diameter) and the bulb of the flask is held about 10 cm. (4 inches) away from the mercury tube of the lamp. Place a sheet of aluminum foil about 7.5 cm. (3 inches) below the flask being irradiated, in order to reflect the light back toward the sample. Agitate the sample every 15 minutes by gently tapping the flask, for example, with a pencil. Allow the sample to heat up as much as the lamp will heat it, provided that the temperature does not reach the boiling point of the solvent being used.

Irradiate until the vitamin A has been destroyed. Under the authors' conditions, approximately 2.5 hours have been required for complete destruction of the vitamin A contained in this concentration of margarine fat unsaponifiable. Destruction of vitamin A can be estimated by the Carr-Price test, and the time of irradiation required for the ultraviolet lamp in use can be established spectrophotometrically by irradiating until there is no further decrease in absorption of the irradited sample at 3280 Å.

After cooling to about 21° C. (70° F.), filter the irradiated solution, which must be clear and colorless, and determine vitamin A in the nonirradiated solution by means of the spectrophotometer (1-cm. cells), using the ultraviolet irradiated solution as a control. With the Hilger spectrophotometer, expose the plates at density settings ranging from 0 to 1.50 in increments of 0.05, with the exposure time graduated up to about 2 seconds on Eastman No. 33 plates. These plates are satisfactorily developed with Eastman D72, diluted 1 to 2. Considerable time can be saved, without any sacrifice in accuracy, by using a Beckman spectrophotometer. For this instrument, 1-cm. square type Corex absorption cells are satisfactory. A tungsten lamp instead of a hydrogen discharge tube can be satisfactorily used as a source of ultraviolet light.

Read the match point (or density) at 3280 Å., and calculate the $E^{1\%}_{1\ cm.}$ value of the sample under test. The difference in absorption at 3280 Å., between the nonirradiated and irradiated sample, is a measure of the vitamin A content of the sample.

This method has been successfully used for determining the vitamin A content of a variety of domestic vegetable oil margarines, but no attempts have been made to apply the method to animal fat margarines or to coconut oil type margarines.

Calculations

$$\frac{\text{Match point (or density) at 3280 Å.}}{40} \times 2140 \times 454 =$$

U.S.P. units of vitamin A per pound of margarine fat

$$\frac{\text{\% fat in sample} \times \text{U.S.P. units of vitamin A per pound of butterfat}}{100} =$$

U.S.P. units of vitamin A per pound of margarine

These calculations are based on 2140 as the conversion factor for vitamin A.

Derivation of Conversion Factor of 2140. The conversion factor to be used for converting from $E^{1\%}_{1\ cm.}$ value at 3280 A., to U.S.P. units of vitamin A per gram of oil, was determined for the instruments employed by use of the U.S.P. standard of reference cod liver oil. The method, consisting of $E^{1\%}_{1\ cm.}$ value determinations, made by the instrument being calibrated, on the unsaponifiable fraction of U.S.P. standard of reference cod liver oil containing 3000 U.S.P. units per gram of oil, has been described (*9*). The saponification and extraction procedure used for removal of the unsaponifiable material from the reference cod liver oil, for spectrophotometric study, is a modification of the procedure published by Wilkie (*12*).

Table II shows the results of conversion factor determinations made with the Hilger spectrophotometer, using the current U.S.P. standard of reference cod liver oil containing 1700 U.S.P. vitamin A units per gram of oil. These determinations include results obtained by the use of both cyclohexane and methyl cyclohexane as solvents. In order to minimize the effect of any possible instability of the U.S.P. reference oil, fresh or practically fresh samples of the reference oil were used for each determination as indicated.

Table II. Establishment of Conversion Factor (Hilger Spectrophotometer)

Date of Determination	Per Cent Solution (Original Oil Basis)	Solvent	Match Point at 3280 Å.	$E^{1\%}_{1\ cm.}$ Value	Conversion Factor
2-28-41	1.000	Cyclohexane	0.795	0.795	2138
2-28-41	1.000	Methyl cyclohexane	0.795	0.795	2138
6-12-42	1.000	Methyl cyclohexane	0.790	0.790	2152
8-4-42	1.000	Methyl cyclohexane	0.790	0.790	2152
8-4-42	1.000	Methyl cyclohexane	0.800	0.800	2125
8-18-42	1.000	Methyl cyclohexane	0.795	0.795	2138
				Av.	2137

Conversion factor of 2140 used. Similar results were obtained by use of Beckman spectrophotometer.

Determinations dated 2-28-41, 6-12-42, and 8-4-42 were made on fresh samples of reference oil, not previously opened. Determination dated 8-18-42 was made on same sample used on 8-4-42 after storage for 2-week interim at 7° C. (45° F.) in the dark and under an atmosphere of carbon dioxide.

Expected Accuracy of Method. In order to determine the limit of vitamin A recovery and the degree of reproducibility which might be expected from the use of this method, several lots of margarine were made under controlled conditions. Samples of the oil going into the margarines were taken before the vitamin A–bearing oils were added. The finished margarines were analyzed by the above method and the oils before processing into margarine were analyzed spectrophotometrically as whole oil, using the nonvitaminized but otherwise identical oil sample as a control. Typical results (Table III) show that very close to theoretical recovery can be obtained by this method, and that the results are reproducible to a satisfactory degree.

Correlation with Biological Assays. In order to determine the agreement between the spectrophotometric method and the biological U.S.P. method, several samples of margarine were analyzed by the spectrophotometric method at about the time that portions of the identical prints were being assayed for vitamin A by commercial biological laboratories.

Table III. Reproducibility and Recovery of Vitamin A

Sample No.	Determination No.	Irradiated Unsaponifiable Method	Spectrophotometric Analysis of Whole Oil, Using Nonvitaminized Control Oil	Vitamin A Found by Irradiated Unsaponifiable Method
		U.S.P. units	*U.S.P. units*	%
I	1	15,500	16,100	96
	2	15,100	15,900	95
II	1	16,900	16,900	100
	2	16,700	16,300	102
III	1	15,100	15,500	97
	2	15,700	15,500	101
IV	1	15,300	15,300	100
	2	15,100	15,700	96
			Av.	98.4

All samples used for this comparison were commercial margarines, received in their original containers; included were several different brands of margarine, made by different manufacturers, and also several prints of one single brand of margarine. The data covering the comparison of results obtained by the above method with those obtained by the U.S.P. biological method were built up over a period of approximately one year.

In order to obtain sufficient data to estimate the biological vitamin A potency of these samples with a reasonable degree of accuracy, the commercial biological laboratories employed for this work were instructed, in most instances, to feed the standard of reference cod liver oil and the sample of margarine under test at two different levels. By means of log-dose interpolation curves, plotting weight response *vs.* sample weight fed, the biological

Table IV. Comparison of Results of Spectrophotometric vs. Biological Methods

(Standard of reference oil containing 1700 U.S.P. vitamin A units per gram used)

Sample No.	Brand	Spectrophotometric Method: Determination No.	Spectrophotometric Method: U.S.P. units per pound of margarine[a]	U.S.P. Biological Method: No. of Levels Fed: U.S.P. reference oil	No. of Levels Fed: Sample	Daily Dose: U.S.P. reference oil	Daily Dose: Sample	Average Gain Weight On: reference oil	Average Gain Weight On: sample	U.S.P. units vitamin A per pound of margarine, average by log-dose interpolation curves[a]
						Mg.	*Mg.*	*Grams*	*Grams*	
1	A	1	9,700			0.88	56.7	28.1	23.1	
		2	9,500	2	2	..	..	..	..	10,100
		3	9,300			1.47	94.5	36.1	35.0	
		Av.	9,500							
2	B	1	16,100			0.88	34.0	30.3	14.0	
		2	16,100	2	2	..	..	..	..	6,900
		3	15,900			1.47	56.7	39.6	20.9	
		Av.	16,000							
3	C	1	15,300			0.882	53.4	19.9	28.9	
		2	15,100	2	2	..	..	..	..	17,500
		3	14,600			1.176	69.8	29.0	40.0	
		Av.	15,000							
4	D	1	9,700			0.882	82.5	19.9	38.9	
		2	8,900	2	2	..	..	..	..	15,300
		3	9,100			1.176	113.5	29.0	51.3	
		Av.	9,200							
5	E	1	16,300			1.176	100.9	37.0	51.9	
		2	16,100	1	1	..	..	..	..	Over 9,000
		Av.	16,200							
6	E	1	11,500			0.88	45.4	29.9	27.9	
		2	11,300	2	2	1.47	75.6	44.2	40.3	13,500
		Av.	11,400							
7	E	1	14,000			0.88	45.4	28.5	34.3	
		2	12,800	2	2	1.47	75.7	51.1	38.4	14,200
		Av.	13,400							
8	E	1	16,100			0.882	..	19.9	..	
		2	15,900	2	1	1.176	100.9	29.0	57.0	21,400
		Av.	16,000							
9	E	1	15,200			0.88	45.4	30.3	28.3	
				2	2	1.47	75.6	48.2	35.0	12,300
10	E	1	12,500			0.88	45.4	40.3	37.4	
				2	2	1.47	75.6	57.3	43.3	11,800
11	E	1	13,400			0.88	45.4	31.4	31.8	
		2	14,000	2	2	..	..	..	..	17,200
		3	14,000			1.47	75.6	38.6	41.9	
		Av.	13,800							
12	E	1	14,200	1	1	1.176	100.9	35.5	47.3	Over 9,000
13	E	1	13,400	2	2	0.88	45.4	30.2	37.8	18,700
						1.47	75.6	43.8	47.8	
14	E	1	16,500			0.88	45.4	28.9	32.8	
				2	2	1.47	75.6	44.3	55.1	18,300
Av. (excluding Nos. 5 and 12, on which only single level bio-tests are available)			13,491	..	..	..	..	..	..	14,766

[a] Results rounded off to nearest 100 units.

vitamin A potency was estimated as the average of the response from the levels fed. All biological tests were made by the U.S.P. method. Two commercial biological laboratories, Food Research Laboratories, Long Island City, N. Y., and the Laboratory of Vitamin Technology, Chicago, Ill., carried out the biological tests. However, each sample was bio-tested by only one of the biological laboratories.

The comparative results, shown in Table IV, demonstrated that consistent and reproducible results are obtained by the spectrophotometric method; these results are in as reasonable agreement with the U.S.P. biological method as could be expected when the known variation in results obtained by the biological method is considered (*11*). It is significant that while in this comparison the biological method gave somewhat higher results than the spectrophotometric method on some samples, the reverse was true on other samples, and the average of all the samples analyzed by the spectrophotometric method is in very close agreement with the average of the same samples tested by the biological method.

A similar condition has been found to exist when comparing biological results obtained by different biological laboratories on identical samples of vitamin A–bearing oils. A separate publication (*11*) covers several years' experience with vitamin A–bearing oils used for enriching margarine and reports biological results obtained by two different laboratories on each of eleven large lots. The average of the results on all samples obtained by the two laboratories agree to within 3% although results on individual samples show variations ranging from 2 to 95%.

Thus some individual variations in results obtained by spectrophotometric and biological methods, as shown in Table IV, must be expected because of the relatively poor reproducibility of the biological method. The results on most individual samples compared by the two methods show reasonably good agreement in vitamin A potency. These results are considered to confirm the validity of the spectrophotometric method described above.

SUMMARY

A spectrophotometric method for the determination of vitamin A in margarine is based on the destruction of vitamin A in a portion of a solution of the unsaponifiable fraction of margarine fat by ultraviolet irradiation, and the use of this devitaminized solution as a control for the spectrophotometric determination of vitamin A in a second portion of the original unsaponifiable solution not irradiated with ultraviolet light. This method gives consistent and reproducible results, and practically complete recovery of the vitamin A contained in margarine. Two satisfactory solvents and two satisfactory instruments for this determination are described. The validity of the method has been demonstrated by comparison with the U.S.P. biological method on identical samples, by feeding both the sample under test and the

U.S.P. standard of reference oil at multiple levels and estimating the biological vitamin A potency from log-dose interpolation curves.

ACKNOWLEDGMENT

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Qualitative Differentiation of the Methylcarbinols and Methyl Ketones

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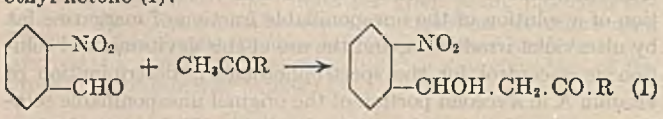
Compounds yielding positive Lieben iodoform reactions may be either methyl ketones or methylcarbinols. On reaction with a reagent containing o-nitrobenzaldehyde in alkaline solution, only the methyl ketones will form indigo and may thus be distinguished readily from the methylcarbinols.

IN THE course of a recent investigation, a simple and rapid method was needed for the qualitative differentiation of methyl ketones and methylcarbinols. The valuable Lieben iodoform reaction (*7*) has long been used to detect the grouping CH_3CO— when joined to a hydrogen atom, or to a carbon atom which does not carry highly activated hydrogen atoms, or groups capable of exerting excessively high steric hindrance. However, the corresponding methylcarbinols (CH_3CHOH—) will also give the Lieben reaction, owing undoubtedly to their prior oxidation by the alkaline hypoiodite reagent to the methyl ketones. Thus, while methyl ketones and methylcarbinols are readily identifiable by the Lieben reaction, there is no simple means of differentiating the two groups.

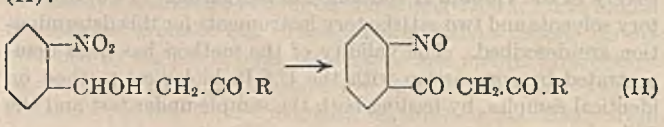
The von Baeyer–Drewsen synthesis of indigo (*1*, *6*), at one time practiced on a commercial scale, is based on a reaction which lends itself readily to such a differential identification technique. *o*-Nitrobenzaldehyde was found by these workers to condense with acetone, acetaldehyde, or pyruvic acid in the presence of alkali, to form indigotin. Feigl, Zappert, and Vasquez (*3*) applied this reaction to six additional methyl ketones (methyl ethyl ketone, methyl heptenone, acetophenone, acetylacetone, diacetyl, and ethyl acetoacetate) and suggested its applicability in detecting other methyl ketones.

Tananescu and his co-workers (*8*, *9*) have suggested that the reaction probably proceeds as follows:

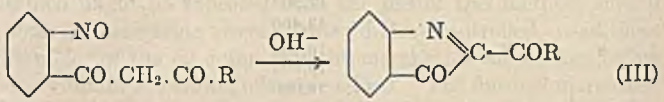
The methyl ketone first condenses with the *o*-nitrobenzaldehyde to form the corresponding β-2-nitrophenyl-β-hydroxyethyl ketone (I):



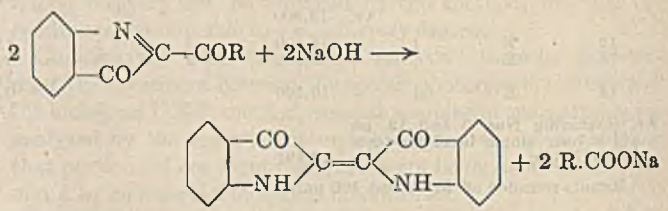
Compound I undergoes an intramolecular oxidation-reduction to form the corresponding β-2-nitrosophenyl-β-ketoethyl ketone (II):



Compound II then cyclizes in the presence of the alkali:



to form Compound III, two moles of which split off R.COOH by hydrolysis and form one mole of indigotin:



As the result of the author's investigation, it was found that a simple qualitative differentiation of methylcarbinols and methyl ketones could be based on the Lieben iodoform reaction in conjunction with the von Baeyer–Drewsen indigo reaction, both being specifically modified for use as an analytical technique. A negative iodoform reaction rules out both methylcarbinols and methyl ketones; a positive iodoform reaction and a negative indigo reaction indicate a methylcarbinol; a positive iodoform reaction and a positive indigo reaction indicate a methyl ketone. This differentiation is specific. When a technical sample of a methylcarbinol was found to give a faint positive indigo reaction, this could invariably be traced to the presence of methyl ketone as an impurity. Scrupulous purification of the methylcarbinol (e.g., via a characteristic crystalline derivative) resulted in a product which reacted entirely in the expected manner.

IODOFORM REACTION

The procedure used for the iodoform reaction was that described by Fuson and Tullock (*5*).

REAGENTS REQUIRED. Dioxane, 10% sodium hydroxide solution, and iodine reagent: 200 grams of potassium iodide and 100 grams of iodine dissolved in 800 cc. of distilled water.

PROCEDURE. About 100 mg. of the compound being tested are placed in a 150 × 16 mm. test tube, 5 cc. of dioxane are added, and the sample is dissolved with shaking. First 1 cc. of 10% sodium hydroxide solution and then the iodine reagent

are added dropwise with shaking until a slight excess of iodine causes a definite dark color which does not disappear on standing. The test tube is now placed in a water bath maintained at 60° C., and the dropwise addition of the iodine reagent is continued until the definite dark color persists as before; but the warming at 60° C. should not last over 2 minutes. The excess of iodine is now removed with a few drops of 10% sodium hydroxide solution, and the test tube is filled with cold water, allowed to stand for 15 minutes, and filtered. The characteristic odor of iodoform is readily distinguishable. As a confirmation, the crystals which are collected on the filter paper are dried at 100° C. for one hour and identified by their melting point. Iodoform melts at 119–121° C.

INDIGO REACTION

Since *o*-nitrobenzaldehyde is not at present obtainable from any domestic source, it may be prepared by nitrating benzaldehyde by the method described by Friedlander and Henriques (*4*) and separating the *o*-nitrobenzaldehyde from the mixture of isomers thus obtained by the method described by Erhart (*2*).

Preparation of Reagent. One hundred grams of finely powdered sodium nitrate are added in small portions to 1 liter of 66° Bé. sulfuric acid, the temperature of the mixture being kept below 20° C. by external cooling. c.p. benzaldehyde (106 grams) is now added to this nitrating mixture in small portions, the temperature being kept below 30–35° C. After all the benzaldehyde has been added, the reaction mixture is cautiously poured into a mixture of 1.5 liters of water and 1.5 kg. of ice. The oily layer of mixed *o*- and *m*-nitrobenzaldehyde is separated by decantation and mixed without heating with 500 cc. of 44% sodium bisulfite solution (sp. gr. 1.37). Six hundred cubic centimeters of water heated to 45° C. are now added to dissolve the magma of crystals and the resultant solution is chilled in a refrigerator at 0° to 5° C. for 48 hours. The copious precipitate of *m*-nitrobenzaldehyde sodium bisulfite which forms is filtered off. To the filtrate is added a saturated sodium carbonate solution to strong alkaline reaction, and the mixture is cooled and extracted with three successive 500-cc. portions of ether. The ether extracts are dried overnight over anhydrous calcium chloride, filtered, and the solvent evaporated off. The residual oil (20 to 25 grams) solidifies on cooling and consists of *o*-nitrobenzaldehyde mixed with smaller amounts of *m*-nitrobenzaldehyde. This product is sufficiently pure for use in the technique described below.

The *o*-nitrobenzaldehyde reagent is prepared by dissolving 5.0 grams of the crystals or the oily product in 100 cc. of 95% ethanol. This reagent solution should be prepared fresh from the undissolved compound at least once a month, and stored in an amber-colored glass-stoppered bottle.

Procedure. About 100 mg. of the compound being tested are dissolved or suspended with vigorous agitation in 5.0 cc. of the *o*-nitrobenzaldehyde reagent, and 1.0 cc. of 10% sodium hydroxide solution is added dropwise. An immediate darkening of the solution will occur (partly due to the formation of dismutation products of *o*-nitrobenzaldehyde). After 60 seconds, a few drops of the solution are placed on a piece of filter paper and allowed to be absorbed. The filter paper is then washed under a stream of tap water and examined. If the brown stain has washed away, the test is negative—i.e., the sample was a methylcarbinol. A positive test—i.e., a methyl ketone—is evidenced by a distinct and unmistakable deposition of indigo-blue dyestuff within the fibers of the filter paper. The "spot" of indigo blue is usually rimmed by a characteristic blue-green ring, which cannot be removed, even by prolonged washing. By comparing the washable brown stains obtained with known methylcarbinols and the permanent blue dye obtained with known methyl ketones, the chemist can soon become highly proficient in distinguishing the two.

This method will detect as little as 1 mg. of methyl ketone in a 100-mg. sample of methylcarbinol. It has been tried with a number of methylcarbinols and methyl ketones. The results obtained may be summarized by classifying the compounds as follows:

Class I. Compounds which give a positive indigo reaction but fail to give a positive iodoform reaction: No compounds were found which fall in this class categorically. Sterically hindered compounds like pinacolone (1) give the indigo reaction more readily than they form iodoform, but these belong properly in Class III.

Class II. Compounds which give a negative indigo reaction and a positive iodoform reaction: ethanol (2), isopropanol (3), methylethylcarbinol (4), methyl-*n*-propylcarbinol (5), methylisopropylcarbinol (6), methyl-*n*-butylcarbinol (7), methylisobutylcarbinol (8), methyl-*n*-amylcarbinol (9), methylisoamylcarbinol (10), methyl-*n*-hexylcarbinol (11), methylisohexylcarbinol (12), butandiol - 2,3 (13), benzylmethylcarbinol (14), 1 - phenylpropandiol-2,3 (15), lactic acid (16), methyl lactate (17), and ethyl lactate (18).

This class, therefore, comprises only the methylcarbinols—i.e., the series of compounds characterized by the grouping CH_3.-CHOH— joined to a hydrogen atom or to a carbon atom which does not carry groups that exert an excessively great steric hindrance.

Class III. Compounds which give a positive indigo reaction and a positive iodoform reaction: acetaldehyde (19), acetone (20), methyl ethyl ketone (21), methyl *n*-propyl ketone (22), methyl isopropyl ketone (23), methyl *n*-butyl ketone (24), methyl isobutyl ketone (25), methyl *n*-amyl ketone (26), methyl isoamyl ketone (27), methyl *n*-hexyl ketone (28), methyl isohexyl ketone (29) acetoin (30), diacetyl (31), phenylacetone (32), 1-phenylpropanol-1, one-2 (33), pyruvic acid (34), methyl pyruvate (35), ethyl pyruvate (36), methyl cyclohexylketone (37), benzyl acetone (38), acetophenone (39), methyl *p*-tolyl ketone (40), *p*-chloroacetophenone (41), *p*-bromoacetophenone (42), methyl *p*-anisyl ketone (43), 2,4-dimethoxyacetophenone (44), *o*-hydroxyacetophenone (45), *m*-hydroxyacetophenone (46), *p*-hydroxyacetophenone (47), 3-methoxy-4-hydroxyacetophenone (48), *o*-nitroacetophenone (49), *o*-aminoacetophenone (50), 2-aceto-1-naphthoxyacetic acid (51), mesityl oxide (52), benzalacetone (53), salicylalacetone (54), vanillalacetone (55), *p*-hydroxybenzalacetone (56), furfuralacetone (57), acetylacetone (58), acetonylacetone (59), benzoylacetone (60), levulinic acid (61), methyl levulinate (62), ethyl levulinate (63), ethyl acetoacetate (64), α-acetyl-γ-butyrolactone (65), pentanol-1-one-4 (66), 5-diethylaminopentanone-2 (67), methylheptenone (68), β-ionone (69), α-ionone (70), methyl vinyl ketone (71).

This class, therefore, comprises only the methyl ketones—i.e., the series of compounds characterized by the grouping $CH_3.CO$— joined to a hydrogen atom or to a carbon atom which does not carry groups that exert an excessively great hindrance.

Interfering compounds in the iodoform reaction are usually (*a*) primary amines which are oxidized to methyl ketones by the alkaline hypoiodite reagent (such as α-aminoisobutyric acid, α-phenylethylamine, 2-aminoheptane, 2-aminohexane, isopropyl amine, etc.), (*b*) esters of ethanol and secondary alcohols which are hydrolyzed by the alkaline reagent to compounds of Class II (such as ethyl acetate, ethyl propionate, diethylphthalate, diethyladipate, *sec*-butyl acetate, *sec*-amyl acetate, isopropylacetate, etc.), and (*c*) oximes which are hydrolyzed to methyl ketones (such as acetoxime, acetophenone oxime, acetone oxime, methyl ethyl ketoxime).

The only compounds which have been found to interfere in the indigo reaction are those which are readily hydrolyzed by the alkaline reagent to compounds of Class III. These are (*a*) halogen derivatives (such as ethylidene chloride, 2,2-dibromopropane, 2,2-dibromobutane, etc.), (*b*) acetals (such as acetaldehyde alcoholate, acetal), (*c*) oximes (such as acetoxime, acetophenone oxime, acetone oxime, methyl ethyl ketoxime, etc.), and (*d*) bisulfite addition products (such as acetaldehyde sodium bisulfite, acetone sodium bisulfite, etc.).

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Pigment Determination in Carbon Black and Iron Blue Paints

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A simple, rapid, and accurate method for quantitative separation of very fine pigments from oil and varnish vehicles is based upon the fact that certain materials yield light, flocculent precipitates which settle rapidly, carrying along the pigment that is present.

A RELIABLE method for accurately separating very small particle size pigments in oil (*3*, *5*) and varnish (*1*, *2*) vehicles has never been satisfactorily worked out. Carbon black, iron blue, chrome greens containing iron blue, and finely ground whites are the most common causes of trouble. It is sometimes possible to filter the fine pigment through a very fine filtering medium but further trouble is experienced because the filter is clogged by the fine particles. Although in some cases it may not be necessary to make a perfect separation and some of the pigment may be passed through the filter, in other cases an exact determination and a clear filtrate are required. The basic principle of the method described in this article can be applied to any pigment-vehicle combination; it is only necessary to find the proper settling agent to collect the pigment and the proper solvent to dissolve the vehicle and to precipitate the settling agent on the pigment.

Since the pigment particles are too small to be retained on the filtering surface, it is necessary to collect them by adding an agent (nitrocellulose, for example) that when precipitated will carry along with it the finely divided pigment. Two methods are outlined in this article; the principle of each is the same; the methods differ only in application and procedure. The first method, which depends upon nitrocellulose to collect the pigment, can be applied to all pigments; it has the disadvantage that the extracted pigment will contain nitrocellulose, although the vehicle will be free from impurities. This method involves no chemical reaction upon any pigment and can be used on nearly any vehicle except emulsion vehicles or poorly soluble vehicles. Certain vehicles, such as run Congo, short oil alkyds, ester gum, and maleic resins sometimes require special treatment, which consists of redissolving and then reprecipitating the nitrocellulose in order to obtain complete extraction of the vehicle.

The second method makes use of a glyceryl phthalate varnish (the phthalate is added if not already present) as the settling agent, precipitated on the pigment in the form of potassium phthalate. Since alcoholic potassium hydroxide is used in this reaction, no alkali-soluble pigments such as lead chromate pigments can be present. Its principal application is to carbon black and other insoluble pigments, to emulsion vehicles, and to poorly soluble vehicles that cannot be determined by other methods. It is based upon the ordnance method for alkyd resin determination (*4*) and is very accurate.

REAGENT

NITROCELLULOSE SOLUTION. Add 20 parts by weight of the dry R.S. 0.25-second nitrocellulose to 80 parts by weight of ethyl acetate. After solution is complete, determine the exact nonvolatile content of the solution.

PROCEDURE

METHOD 1 (Nitrocellulose Method). Weigh accurately a 2- or 3-gram sample of the paint or enamel and pour into a tared 50-cc. centrifuge tube. Add 10 cc. of ethyl acetate (85% ester), and stir until sample and ester are completely mixed. Pour into this mixture a weighed sample (about 3 grams) of the nitrocellulose solution, and stir until a smooth mixture is obtained. Precipitate this mixture by adding slowly, drop by drop, about 30 cc. of high-solvency aromatic Hi-Flash naphtha, stirring rapidly during the addition. Then place the tube in a water bath and raise to a temperature of 180° F.; hold at this temperature overnight or until the ester has completely evaporated. At the end of this time, remove from the water bath, cool to room temperature, refill to top with more high-flash naphtha, and centrifuge until the top liquid is clear. Pour off the clear liquid and refill with benzene, place in a water bath at 140° F., and allow to stand for about 1 hour. Cool and centrifuge as before. Repeat the centrifuging with the benzene as before and finally wash with petroleum ether, omitting the water bath. Dry at 150° F. to constant weight. Calculate pigment by subtracting the known amount of nitrocellulose from the total weight.

METHOD 2 (Phthalate Method). Weigh accurately 7 or 8 grams of the sample into a 250-cc. stoppered Erlenmeyer flask and add 5 cc. of butyl Cellosolve (ethylene glycol monobutyl ether) to aid compatibility of the vehicle. Shake the flask carefully, so that the sample and Cellosolve are thoroughly mixed. Add 3 or 4 cc. of a long oil glyceryl phthalate varnish (unless already present) and shake until a smooth mixture is obtained. It is not necessary to weigh the glyceryl phthalate varnish, as it does not enter into the calculation. In case a smooth mixture does not result at this point, add more Cellosolve slowly until a smooth mixture is obtained. Next add 125 cc. of 0.6 *N* potassium hydroxide in alcohol (5 grams of potassium hydroxide) and shake thoroughly. Stopper and allow to stand about 3 hours at 130° F. to allow for precipitation. At the end of this time remove from oven, cool to room temperature, add 50 cc. of ethyl ether, and restopper; allow to stand at least 1 hour. Filter through a tared dry filter paper (Whatman No. 32, for example), washing with 50 cc. of alcohol-ether (1 to 1) mixture, using five 10-cc. portions. Dry in an oven at 200° F. for 10 or 15 minutes, remove from oven, and replace in filtering funnel. Wash with warm water (160° F.) until all the soluble potassium phthalate is dissolved and the pigment is washed free of the phthalate. Make the final washing with ethyl alcohol to shorten the drying period. Dry in an oven at 220° F. to constant weight.

Table I. Pigment Determinations in Different Vehicles

Pigment	Vehicle	Method	Pigment Theoretical, %	Pigment Determined, %
Carbon black	Congo varnish	Phthalate	6.60	6.60
White pigments	Emulsion	Phthalate	50.0	46.3
Iron oxide	Alkyd	Phthalate	43.7	43.7
Iron blue	Rosin varnish	Nitrocellulose	51.3	51.7
Carbon black	Linseed oil	Nitrocellulose	3.20	3.20
Carbon black	Alkyd varnish	Nitrocellulose	3.0	3.4

Because of the great variety of enamel and paint vehicles, many different types of solubilities are encountered. Table I shows some applications of the two methods and the results obtained. Since the samples were all commercial grades and subject to slight variations, the results are reasonably close. The use of nitrocellulose limits the vehicle solubility to esters and aromatic hydrocarbons. However, the number of cellulose derivatives is large enough to cover practically all vehicles. Ethyl cellulose, benzyl cellulose, cellulose acetate, cellulose acetate butyrate, and other agents may be used for vehicles in which nitrocellulose is not satisfactory. The choice of liquids is very extensive. For solvents there are esters, ketones, and ethers; for precipitating liquids there are aromatic and aliphatic hydrocarbons, terpenes, and alcohols. The choice is determined by the ability of the cellulose product to precipitate on the pigment while the vehicle remains in solution.

The precipitation method of pigment separation results in considerable time-saving in most cases. The material filters very rapidly on a vacuum filter and does not clog the filter. Actually only about 0.5 to 0.75 hour is the total time necessary for the complete operation, including weighing, filtering, and washing. There are many variations in the procedure outlined in this article and the proper application of the general principles of the method should yield accurate results in most cases.

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A Device for Renewing the Filter-Cake Surface in Small-Scale Vacuum Filtrations

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THIS note describes a device which has been used successfully on a laboratory and semipilot-plant scale to expedite the vacuum filtration of alkaline aqueous dispersions of vegetable protein extracted from peanut and cottonseed meals. Its effectiveness suggests that it might prove useful in clarifying various gummy or colloidal solutions. It can be easily and inexpensively constructed.

The device consists of a hand-operated rotary scraper which can be used with either a table-top Büchner funnel or a stoneware suction filter. By means of the scraper fine colloidal and gummy materials which accumulate on and clog the surface of the filter cake can be periodically removed during filtration along with a thin layer of the filter aid used. By means of this periodic scraping the filter cake surface can be renewed as frequently as is necessary to maintain the filtration rate at a maximum.

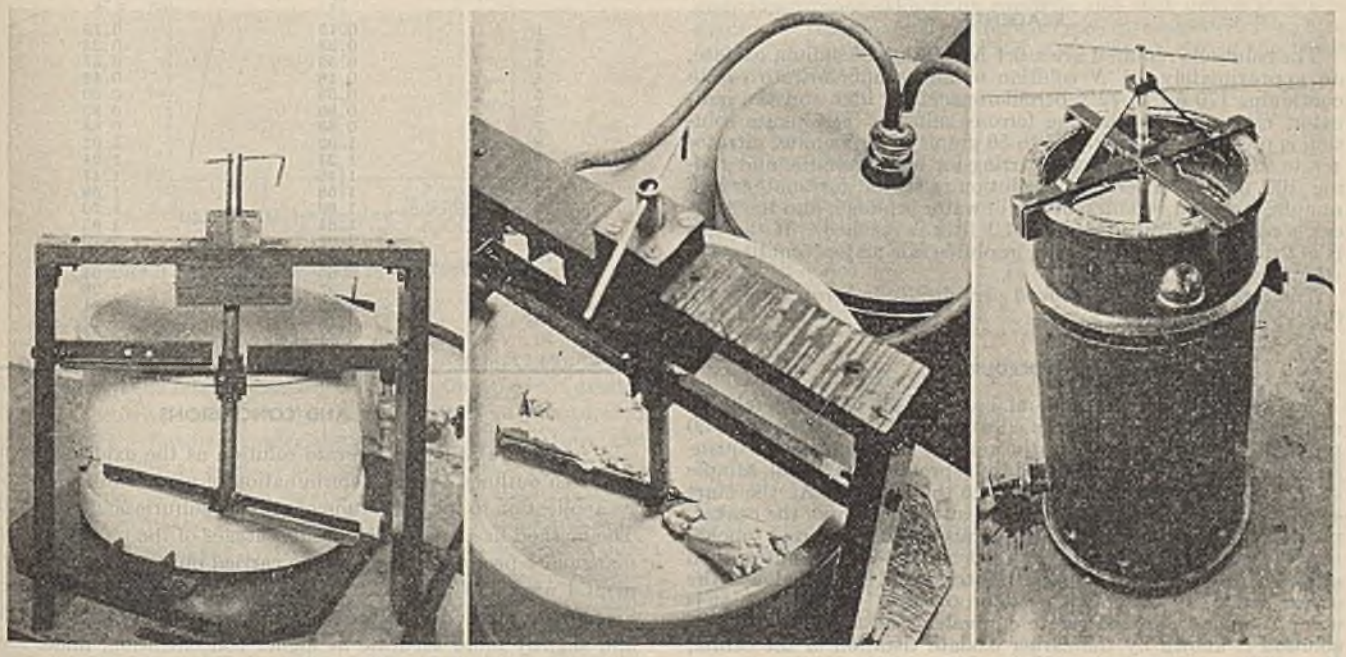

Figure 1. Table-Top Büchner Funnel and Scraper Assembly, Assembled Filtering Unit, and Stoneware Suction Filter with Scraper Attachment

The table-top Büchner funnel, the scraper assembly and plywood base to which it is bolted are illustrated in Figure 1 (left). The assembled unit, including a protected glass bottle for collecting the filtrate is illustrated in Figure 1 (center). The scraper blades are of 1 × 0.125 inch stainless sheet steel bent at an angle of 45° and welded to a shaft of 0.5-inch stainless steel pipe. The lower edges of the blades are sharpened. The shaft is supported by a bearing attached to the wooden frame by two pieces of angle iron and a threaded bronze block screwed to the top of the frame. Forty micrometer threads per inch are cut on the shaft and in the block. Although a much thinner cake is ordinarily used, it was considered desirable to cut enough threads on the shaft to allow the scraper to travel through about 3 inches of cake. As the clogged filter aid and gummy material are scraped from the surface they collect in a cone-shaped mass (Figure 1, center) which can be removed whenever a sufficiently large amount accumulates.

A similar scraper designed for use with a stoneware suction filter is shown at the right. The lower bearing is threaded and the scraper assembly is fastened to the top of the filter by screw clamps which are welded to a support resting on the filter.

Better results are obtained when no more vacuum is employed than is necessary to maintain a steady flow through the filter. More rapid filtration of alkaline solutions can be obtained when the filter aid is supported by a glass filter cloth instead of a cotton cloth or filter paper. This is probably due to the fact that glass fibers, unlike cellulose fibers, do not swell and slow the rate of filtration when in contact with alkaline solutions.

The principle of this laboratory filter cake scraper is similar to that used on one type of commercial rotary drum filter manufactured in this country, in that the clogged filter-aid surface is removed by scraping action.

Determination of Nitrogen Dioxide by Cerate Oxidimetry

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IN THE manufacture of nitric acid by the Ostwald process and its modifications, the amount of nitrogen dioxide in the final acid must be determined. In addition to the effect of nitrogen dioxide in industrial uses of the acid, the amount present is a measure of the efficiency of the air bleach, and represents a waste of the gas. The amount of nitrosyl sulfuric acid in a mixed acid must be determined in order to establish the percentage of the nitric and sulfuric acids present.

The standard method (*2*) of nitrogen dioxide determination, used in most plants where such work is needed, is titration of a 10-ml. sample with 0.2 *N* potassium permanganate until a pink color persists for 3 minutes. The method is accurate only with careful manipulation of the sample. In actual control practice loss of nitrogen dioxide from the solution under even the best of conditions, speed of addition of potassium permanganate, amount of stirring of the solution while titrating, and individual definition of what constitutes a permanent pink color, as well as the error caused when a rushed analyst shortens the 3-minute period to 2 minutes or even 1—all contribute to inaccuracy. Nitrosyl sulfuric acid is also determined by permanganate titration, using either a 10-ml. sample or an aliquot thereof.

The many published works, and especially those of Smith (*1*, *3*), amply prove the advantages of cerimetry in regard to stability and oxidizing power of the solution, reversibility of the reaction, and lack of side reactions and interferences. These advantages have been retained in developing a method for determination of nitrogen dioxide that is accurate and rapid, even in the hands of an inexperienced analyst.

REAGENTS

The solutions required are a 0.1 *N* solution of sodium oxalate, an approximately 0.1 *N* solution of ammonium nitrato cerate containing 170 ml. of 72% perchloric acid per liter and the indicator, nitro-*o*-phenanthroline ferrous sulfate. The cerate solution is prepared by adding 55 to 56 grams of ammonium nitratocerate to the perchloric acid, stirring for half a minute, and adding 100 ml. of water. The solution is stirred for another half minute, a second 100-ml. portion of water is added, and the process is repeated until a volume of 1 liter is reached. If the salt is not dissolved in this way, an insoluble salt may precipititate in a few days' standing.

The cerate solution is checked periodically in terms of exactly 0.1 *N* sodium oxalate.

PROCEDURE

To 100 ml. of water in a 250-ml. beaker, 5 ml. of 72% perchloric acid are added. To this acidified solution in most cases 25 to 50 ml. of 0.1 *N* cerate are added, the amount varying with the quantity of nitrogen dioxide expected to be present. A 10-ml. sample of the acid is pipetted accurately into the beaker. At the start of delivery, the pipet tip should be near the bottom of the beaker; near the end of delivery, the tip is raised until it barely touches the surface of the solution. It is let stand 1 or 2 minutes, then stirred slowly. Two to 3 drops of nitroferroin are added, and the excess cerate is titrated with sodium oxalate. The end point is sharp. Since the exact cerate equivalence in terms of a 0.1 *N* solution is known by the earlier oxalate titration of the cerate, the milliliters of cerate actually used in oxidation of the nitrogen dioxide will be the equivalence figure minus the milliliters of 0.1 *N* oxalate used in titration of the residual cerate. The percentage of nitrogen dioxide may be calculated by the usual equations, recognizing that 0.1 *N* solutions have been used in place of 0.2 *N* potassium permanganate.

EXPERIMENTAL

The method was carefully checked against the potassium permanganate procedure, using a series of acid samples containing varying amounts of nitrogen dioxide. Extreme care was taken to assure maximum accuracy in the permanganate determination. The potassium permanganate solution was floated upon the surface of the acid water, and not stirred until very close to the end point, when very slow stirring was initiated. The prescribed 3-minute period was taken to assure permanency of the pink potassium permanganate color.

Table I presents data comparing percentages of nitrogen dioxide as determined by the potassium permanganate and cerate procedures.

The determinations by the cerate method were completed in much less time than those by the permanganate procedure. The results compare favorably. In the higher percentages of nitrogen dioxide it is believed that results by the cerate procedure are more accurate than by the permanganate, because of sharper end point and absence of opportunity for possible loss of nitrogen dioxide, since the gas is always in contact with an excess of oxidizing agent.

No attempt was made to extend the method directly to the nitrosyl sulfuric acid determination. Since the potassium permanganate titration for this is identical with that for nitrogen dioxide, the writer can see no reason why the cerate titration should not be entirely satisfactory for this estimation.

Table I. Determination of Nitrogen Dioxide

(In approximately 60% nitric acid)

Sample No.	Nitrogen Dioxide Found	
	Permanganate method	Cerate method
	%	%
1	0.15	0.15
2	0.28	0.28
3	0.36	0.37
4	0.48	0.48
5	0.59	0.60
6	0.80	0.80
7	0.88	0.88
8	1.02	1.02
9	1.24	1.24
10	1.46	1.47
11	1.58	1.59
12	1.69	1.70
13	1.84	1.85
14	2.09	2.10
15	2.26	2.27
16	2.63	2.64
17	2.97	2.98
18	3.49	3.51
19	4.16	4.17
20	5.34	5.36

SUMMARY AND CONCLUSIONS

A procedure utilizing a cerate solution as the oxidizing agent has been outlined for the determination of nitrogen dioxide, and its application to the estimation of nitrosyl sulfuric acid suggested. The method has none of the disadvantages of the potassium permanganate procedure and may be carried out rapidly. Experimental work has proved it to be fully as accurate as the permanganate method in lower concentrations of nitrogen dioxide and slightly more accurate at higher concentration, under conditions in which extreme care was taken with the permanganate procedure. Under rushed control laboratory conditions, the cerate procedure is much more accurate than the permanganate.

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Determining Hygroscopicity of Fertilizers

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A rapid method is described for determining the hygroscopicity of fertilizers by measuring the relative humidity of the air in equilibrium with the mixtures. From curves showing these values against the moisture contents, the storage quality of fertilizers can be determined.

THE hygroscopicity of a fertilizer, or its tendency to absorb moisture, determines to a large extent whether it will remain drillable under humid conditions and whether it will cake on storage. A rapid and accurate method for measuring the hygroscopicity of a fertilizer would, therefore, be a means of predicting the behavior of the fertilizer under practical conditions.

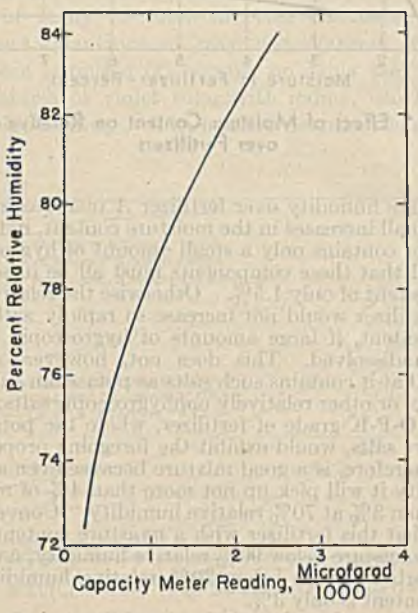

Figure 1. Relative Humidity-Resistance Curve

A fertilizer material will absorb moisture from the surrounding atmosphere if the relative humidity of that atmosphere is greater than that corresponding to the vapor pressure of a saturated solution of the fertilizer material at the same temperature, and will lose moisture at relative humidities below that value. The aqueous vapor pressure of a saturated solution is thus a measure of the hygroscopicity of the solid in question. Adams and Merz (*1*) have determined the hygroscopicity of a large number of pure fertilizer compounds and their mixtures by measuring the relative humidity over their saturated solutions by means of an isotenioscope. This method, however, requires the evaporation of a large quantity of water from the liquid phase and is, therefore, not applicable to mixed fertilizers which generally contain only a small amount of moisture. The withdrawal of much water would disturb the equilibria in such systems.

Ordinarily, when it is desired to determine at what relative humidity a fertilizer mixture begins to take up moisture, a number of tared samples are exposed to various known relative humidities in controlled-humidity chambers. The relative humidity at which the sample just begins to gain weight is noted. This is the threshold value above which the fertilizer mixture will absorb moisture and below which it will not. This method, however, will not always give the exact values because it is impractical to prepare humidity chambers to cover the entire humidity range in narrow intervals. The method described in this paper, however, permits accurate determinations of the hygroscopicity of fertilizers in about 30 minutes with no weighings and without the humidity chambers required for the usual method.

Over each fertilizer mixture, there must exist a partial aqueous vapor pressure corresponding to the vapor pressure of the complex solution contained in that particular fertilizer mixture. If, therefore, a fertilizer mixture is kept in a closed container until equilibrium is established at a desired temperature, and a method is found to determine the relative humidity over the sample, a measure of the hygroscopicity of the sample will have been obtained.

For measuring the relative humidity in a small enclosed space, the electric hygrometer as developed by Dunmore (*2*) of the National Bureau of Standards, was found to be most satisfactory. The electric hygrometer unit consists of a moisture-sensitive film containing lithium chloride on a bifilar coil of palladium wire wound on a thin-walled polystyrene tube. The resistance between the two terminals of the electric hygrometer varies with the relative humidity to which the unit is exposed. The humidity-resistance calibration curve for one such unit is shown in Figure 1. It requires five units with moisture-sensitive films containing various amounts of lithium chloride to cover the whole humidity range.

APPARATUS

The arrangement of the apparatus used for this determination is very simple, as shown in Figure 2. *A* is a 16-ounce bottle about 1/3 full of a fertilizer mixture, *B*. *C* is an electric hygrometer unit. *D* and *D'* are terminals of the unit coming through the two holes in the rubber stopper. These terminals are soldered to copper tips *F* and *F'* on top of the glass rods, *G* and *G'*, coming through the same openings in the rubber stopper to hold the unit in place.

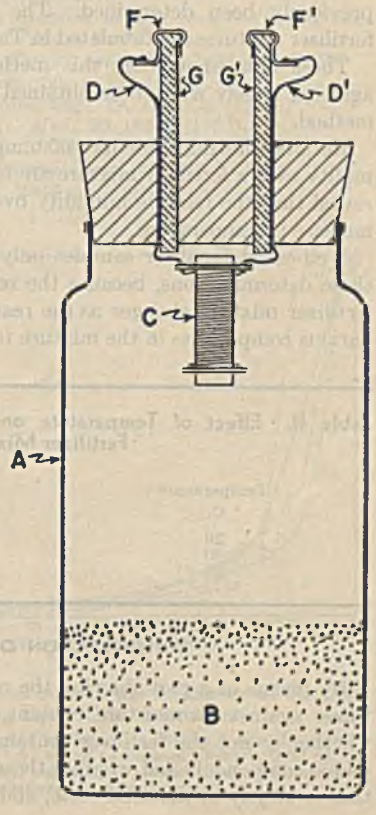

Figure 2. Electric Hygrometer Unit Assembly

Table I. Relative Humidity over Fertilizer Mixtures at 30° C.

Fertilizer Mixture No.	Relative Humidity over Sample by Electric Hygrometer Method	% Moisture Absorbed at Equilibrium at Relative Humidity of 59.4%	65.2%	72.5%
1	60.2	−0.27	5.57	14.36
2	59.7	0.01	11.66	23.32
3	59.1	−0.34	7.26	14.32
4	65.5	−1.31	0.40	14.96
5	65.7	−1.59	−0.18	9.70
6	72.6	−1.67	−1.21	−0.55
7	55.6	10.23	17.08	26.07
8	< 51.4	9.04	12.99	22.34
9	64.3	−1.20	2.18	15.65
10	61.9	−0.89	5.12	12.95
11	61.4	−2.44	3.20	11.17
12	67.6	−2.23	−1.38	10.44
13	71.6	−2.31	−1.82	5.43
14	73.2	−1.99	−1.77	−1.28

PROCEDURE

The procedure for making a determination is also very simple. An electric hygrometer of the proper humidity range is inserted in the bottle containing the fertilizer sample to be tested, as shown in Figure 2, and the whole assembly is allowed to stand for about 20 minutes to come to equilibrium. The resistance between the electric hygrometer terminals is then measured by connecting F and F' to a resistance meter (not shown), or a Weston Model 764 capacity meter. The capacity meter may be used as an ohmmeter, since it is measuring only ohmic resistance in a nonreactive circuit (*2*). A steady reading of the meter signifies that equilibrium has been established. The relative humidity over the fertilizer sample is then read off from the calibration curve for the unit used, like the one shown in Figure 1.

These measurements can be conveniently made in a constant-temperature room, or carried out elsewhere, provided a thermometer is inserted through the rubber stopper to record the temperature of the sample at the time of the measurement. Enough time should be given for the samples to come to equilibrium.

EXPERIMENTAL RESULTS

In order to test the validity of this method, the relative humidities over a number of well-cured fertilizer mixtures were measured at 30° C. by the electric hygrometer method. The equilibrium moisture absorption values of these fertilizers at 59.4, 65.2, and 72.5% relative humidities at the same temperature had previously been determined. The results obtained for these fertilizer mixtures are tabulated in Table I.

These data show that this method gives consistent results agreeing closely with those obtained by the moisture-absorption method.

Results showing the effect of temperature on the relative humidity over a fertilizer mixture are tabulated in Table II. They reveal that the relative humidity over a fertilizer increases with increase in temperature.

Well-cured fertilizer samples only should be used in making these determinations, because the relative humidity over a raw fertilizer mixture changes as the reactions progress between the various components in the mixture to form more stable salts (*3*).

Table II. Effect of Temperature on Relative Humidity over a Fertilizer Mixture

Temperature ° C.	Relative Humidity over Fertilizer %
20	62.9
30	68.2
45	73.0

INTERPRETATION OF RESULTS

By means of a plot showing the relative humidity over a fertilizer against its moisture content, it is possible to judge (*a*) whether or not the fertilizer contains a large amount of hygroscopic components and whether these components are all in solution or largely in the solid state, and (*b*) the amount of moisture the fertilizer will take up at a given relative humidity and at what relative humidity the absorption of moisture will begin with a given moisture content in the fertilizer.

Figure 3 shows three curves, somewhat idealized, that represent relative humidity conditions over three types of fertilizers designated as A, B, and C. An interpretation of these curves will serve to illustrate the points mentioned above.

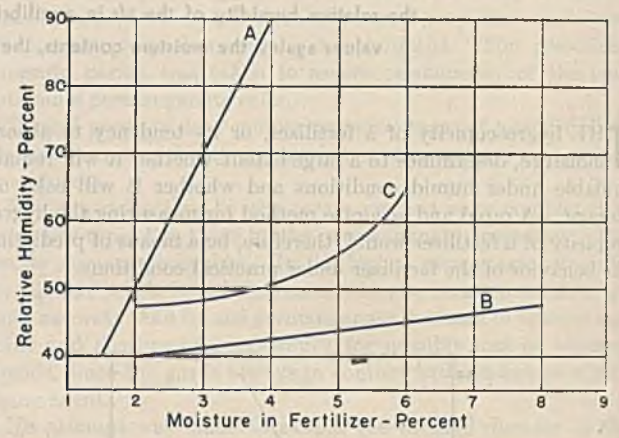

Figure 3. Effect of Moisture Content on Relative Humidity over Fertilizers

The relative humidity over fertilizer A increases rapidly with relatively small increases in the moisture content, indicating that this fertilizer contains only a small amount of hygroscopic components and that these components must all be in solution at a moisture content of only 1.5%. Otherwise the relative humidity over the fertilizer would not increase so rapidly with increasing moisture content, if large amounts of hygroscopic components remained undissolved. This does not, however, exclude the possibility that it contains such salts as potassium sulfate, potassium nitrate, or other relatively nonhygroscopic salts, in the solid state. An O-P-K grade of fertilizer, where the potash is other than manure salts, would exhibit the foregoing properties. Fertilizer A, therefore, is a good mixture because even at 90% relative humidity it will pick up not more than 4% of moisture and not more than 3% at 70% relative humidity. Conversely, curve A reveals that this fertilizer with a moisture content of 4% will not absorb moisture below 90% relative humidity, nor does it begin to absorb moisture below 70% relative humidity when its moisture content is only 3%.

Curve B shows that the relative humidity over fertilizer B increases very slowly as the moisture increases. This means that this fertilizer contains a large amount of very hygroscopic materials and that they are not all in solution at the beginning when the mixture contains 2% of moisture. Fertilizer B, as revealed by the curve, will, therefore, not stand up well under humid conditions because, even when containing 9% of moisture, its relative humidity is still below 45%, and the curve has not even begun to show a break, which means that it still contains hygroscopic substances in the solid state.

Fertilizer C shows little change in relative humidity when its moisture content increases from 2 to 3%, but soon after that curve C starts to turn upward, showing that the more hygroscopic components have all gone into solution and that the solution begins to get more and more dilute with further increases in moisture content. Fertilizer C, intermediate between A and B, is a fairly good mixture because in an atmosphere of 70% relative humidity its moisture content cannot be more than 6%; otherwise the solution present in the fertilizer will have a relative humidity higher than 70%, in which case the fertilizer will lose moisture.

DISCUSSIONS

In actual practice, this type of curves may not always come as regular as these. It is well known that when a fertilizer once gets wet and then dries again, it becomes a little more hygroscopic than the original mixture. This shows up more with mixtures containing soluble components in large granular forms than those having such components in the fine state. On account of this, the curve obtained by introducing moisture to the mixture may

not quite coincide with the curve obtained by removing moisture from the wet mixture. Once equilibrium has been established, however, the values will become constant.

In routine analysis, a single relative humidity measurement is enough to classify a very wet fertilizer having low relative humidity over it as unsatisfactory, because even with such high moisture content, this fertilizer still contains a concentrated solution of its hygroscopic components and some of them may still be in the solid state. On the other hand, a wet fertilizer having high relative humidity over it may be a good one, provided its moisture content can be reduced to a satisfactory value.

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Stable Starch Solution for Dissolved Oxygen Determinations

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NUMEROUS starch solutions for use in iodometric titrations including the Winkler (*15*) determination of dissolved oxygen have been described (*1, 4, 8, 9, 12, 14*), but the preparation of many of these involves elaborate procedures requiring exact quantities of reagents. Various starch solutions have been criticized (*2*) because on aging they frequently produce a reddish or violet color with iodine, which prevents sharp end-point readings. The method described here not only eliminates the need for laboratory conveniences and weighed reagents but yields a starch solution which remains unchanged a year or more.

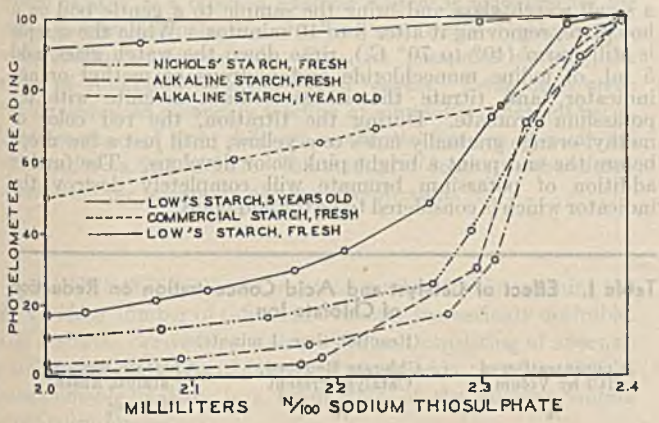

Figure 1. Photelometric Comparisons of Indicator Efficiency of Starches

Free iodine in similar samples was reduced by thiosulfate titration in photelometer to a reading of 92, exactly 0.2 ml. of test starch added, and titration continued to disappearance of blue starch-iodine color.

Method. Advantage is taken of the property of caustic alkali to dissolve the coating on the starch grains without affecting the starch itself (*3, 5, 10, 11, 13*).

A 20% solution of sodium or potassium hydroxide, or the solid caustic, is added with stirring to a suspension of about 2.0 grams of powdered starch in 300 to 400 ml. of water until a thick, sirupy, almost clear solution is obtained. About 30 ml. of 20% potassium hydroxide are required to treat approximately 2 grams of potato starch. The solution is allowed to stand for about 1 hour to ensure complete action by the alkali, and then made neutral or slightly acid with concentrated hydrochloric acid (*6*), using litmus paper as indicator. This product is designated as "alkaline starch". If acidity does not interfere with the proposed titration (the final sample in dissolved oxygen titration by the Winkler method is acidic), 1.0 ml. of glacial acetic acid is added as a preservative.

One lot of this starch solution was used by the writer over a 12-month period without deterioration, mold growth, loss of potency, or production of reddish color in iodometric titrations for dissolved oxygen. The indicator properties of several starch solutions were compared photelometrically by titrating to final end-point partitioned samples of water prepared by the Kemmerer (*7*) method for the iodometric determination of dissolved oxygen, in a Cenco Photelometer (Figures 1 and 2).

This method of preparing starch solution lends itself to use in the field, yields a solution which develops and maintains greater depth of color per unit, and has a sharper end point than any of the starches tested.

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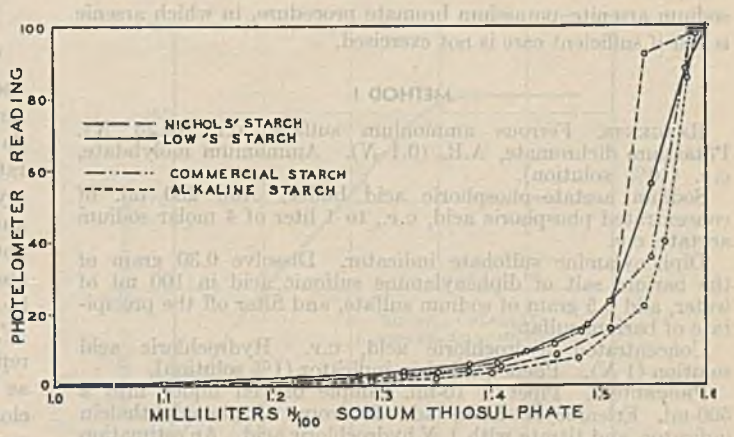

Figure 2. Photelometric Comparisons of Indicator Efficiency of Starches in Quantities Producing Equal Color Intensities

In similar 50-ml. samples free iodine was reduced by thiosulfate titration in photelometer to a reading of 82. Sufficient starch solution (1.51 ml. of commercial Lintner's, 0.60 ml. of Low's, 0.41 ml. of Nichols', 0.26 ml. of alkaline) was added to produce photelometer reading of exactly 0, and titration continued to clear end point.

Rapid Estimation of Chlorate Ion Employing Catalysis

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A method for estimating chlorate in cell liquor which is produced during the manufacture of chlorine consists of reducing the chlorate ion in 40% hydrochloric acid by volume with a standard ferrous ammonium sulfate solution. A few drops of a 10% solution of ammonium molybdate are employed as catalyst. The excess standard ferrous ammonium sulfate is then titrated with standard potassium dichromate, using diphenylamine sulfonate as the redox indicator.

SEVERAL methods for the determination of chlorate ion are described in the literature. Bacho (*1*) reduced the chlorate ion with excess sodium arsenite in hydrochloric acid solution, and then used the potassium bromate method of Györy (*4*) in determining the excess arsenite. Peters and Deutschländer (*6*) employed an arsenite-bromide mixture in a strong hydrochloric acid solution. Osmium tetroxide was suggested as a catalyst by Gleu (*3*). Bölge and Troberg (*2*) reduced the chlorate ion with excess cuprous chloride at 80° C. and finally titrated the excess with standard potassium dichromate. Harvey (*5*) used a ferrous sulfate–potassium iodide system, titrating the liberated iodine with standard sodium thiosulfate.

Essentially two methods are employed at Basic Magnesium, Incorporated, for the determination of sodium chlorate in Hooker cell liquor from the chlorine plant. Method II, described in this paper, is a modified Bacho procedure, which differs from the original method in that refluxing of the sodium arsenite–sodium chlorate mixture is omitted; iodine monochloride is employed as a catalyst in the titration of the excess sodium arsenite by potassium bromate, thus sharpening the end point and making the titration possible at lower than boiling temperatures. Smith (*7*) advocates use of this catalyst in the potentiometric estimation of arsenite by potassium bromate.

A newer procedure for the estimation of chlorate ion, Method I, is being used currently in this laboratory. It consists of reducing the chlorate with an excess of ferrous ammonium sulfate in a strong hydrochloric acid solution, using ammonium molybdate as a catalyst. The excess ferrous ammonium sulfate is titrated with potassium dichromate, using diphenylamine sulfonate as the internal redox indicator.

Method I is preferred because of its high degree of accuracy and precision. It is less sensitive to variable conditions than the sodium arsenite–potassium bromate procedure, in which arsenic is lost if sufficient care is not exercised.

METHOD I

REAGENTS. Ferrous ammonium sulfate, C.P. (0.25 *N*). Potassium dichromate, A.R. (0.1 *N*). Ammonium molybdate, C.P. (10% solution).

Sodium acetate–phosphoric acid buffer. Add 250 ml. of concentrated phosphoric acid, C.P., to 1 liter of 4 molar sodium acetate, C.P.

Diphenylamine sulfonate indicator. Dissolve 0.30 gram of the barium salt of diphenylamine sulfonic acid in 100 ml. of water, add 0.5 gram of sodium sulfate, and filter off the precipitate of barium sulfate.

Concentrated hydrochloric acid, C.P. Hydrochloric acid solution (1 *N*). Phenolphthalein indicator (1% solution).

PROCEDURE. Pipet a 10-ml. sample of cell liquor into a 500-ml. Erlenmeyer flask, add 2 drops of phenolphthalein indicator, and titrate with 1 *N* hydrochloric acid. An estimation of total alkalinity sufficiently accurate for cell liquor chemical control may be made at this point. Add 10 ml. of 0.25 *N* ferrous ammonium sulfate solution, 3 drops of ammonium molybdate catalyst, and 40 ml. of concentrated hydrochloric acid. Allow the mixture to stand 1 minute for complete reaction, then add 20 ml. of phosphoric acid–sodium acetate buffer reagent. Dilute to 200 ml. with distilled water, add 3 drops of diphenylamine sulfonate redox indicator, and titrate with 0.1 *N* potassium dichromate to the purple end point. A correction of 0.05 ml. of dichromate is made for each 6 drops of indicator solution (*8*).

METHOD II

REAGENTS. Sodium arsenite, A.R. (0.1 *N* solution). Potassium bromate, C.P. (0.1 *N* solution).

Iodine monochloride. Dissolve 0.279 gram of pure potassium iodide and 0.178 gram of pure potassium iodate in 250 ml. of water. Add at one time 250 ml. of concentrated hydrochloric acid (sp. gr. 1.19). The resulting solution is 0.005 *M* in iodine monochloride.

Concentrated hydrochloric acid, C.P. Phenolphthalein indicator (1% solution). Methyl orange indicator (0.1% solution).

PROCEDURE. Pipet a 10-ml. sample of cell liquor into a 500-ml. Erlenmeyer flask, add 1 or 2 drops of phenolphthalein, and titrate the sample with 1 *N* hydrochloric acid. An estimation of total alkalinity sufficiently accurate for cell liquor chemical control may be made at this point. Add 30 ml. of standard arsenite solution and 20 ml. of concentrated hydrochloric acid, and dilute to 100 ml. with distilled water. Cover the flask with a small watch glass and bring the sample to a gentle boil on a hot plate, removing it after 8 to 10 minutes. While the sample is still warm (40° to 70° C.), rinse down the watch glass, add 5 ml. of iodine monochloride and 5 drops of methyl orange indicator, and titrate the excess sodium arsenite with 0.1 potassium bromate. During the titration, the red color of methyl orange gradually fades to a yellow, until just a few drops before the end point a bright pink color develops. The further addition of potassium bromate will completely destroy the indicator which is considered to be the end point.

Table I. Effect of Catalyst and Acid Concentration on Reduction of Chlorate Ion

(Reaction time, 1 minute)

Concentration of HCl by Volume	Chlorate Reduced, Catalyst Present	Chlorate Reduced, Catalyst Absent
%	%	%
18	34.99	32.90
31	91.22	82.45
36	98.40	94.61
40	99.70	98.80
50	99.70	98.80

DISCUSSION

Table I illustrates the importance of acid concentration in the determination of chlorate ion, using an excess of standard ferrous ammonium sulfate with and without ammonium molybdate as a catalyst. The standard 0.1 *N* solution of sodium chlorate was prepared from Merck reagent quality sodium chlorate crystals; 10 ml. of this solution were employed. The ferrous ammonium sulfate solution was standardized against the standard potassium dichromate solution in both the presence and the absence of the catalyst. The results indicated no interference by the molybdate.

Table II gives a comparison of Methods I and II. The values, represent the sodium chlorate content of the samples expressed as milliliters of 0.1 *N* solution. In general, the methods are in close agreement, accounting for about 99% of the chlorate ion present. In routine analyses for control purposes this accuracy is entirely adequate.

Hypochlorite in cell liquor is not considered in this discussion since it is present only in microquantities. The hot alkaline liquor of the Hooker cell promotes the formation of chlorate ion.

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Table II. Comparison of Methods I and II on Hooker Cell Liquor

Sample No.	Method I	Method II
1	2.82, 2.84	2.77, 2.72
2	6.45, 6.41	6.32, 6.22
3	16.77, 16.84	16.77, 16.84

SUMMARY

After considerable investigation, it is believed that the most rapid, accurate method for the determination of sodium chlorate in cell liquor produced in chlorine manufacture is the reduction of the chlorate with excess ferrous ammonium sulfate in 40% hydrochloric acid by volume. Ammonium molybdate is used as the catalyst. The method is accurate to within about 1% of the amount of chlorate present. It permits greater precision than the sodium arsenite–potassium bromate method.

The sample of cell liquor for the sodium chlorate estimation may also serve for a total alkalinity determination if standard hydrochloric acid is used in the initial neutralization process.

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An Iodine Number Method for Tall Oil

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The use of pyridine sulfate dibromide in conjunction with mercuric acetate catalyst as a bromine addition reagent is suggested for the iodine number determination of tall oil and similar highly unsaturated, conjugated compounds. Data are presented showing the effects of absorption time and excess reagent. Evidence is given that the undesired secondary reaction of substitution does not occur. Iodine numbers of eight different commercial samples of crude tall oil ranged from 237 to 287. This method of iodine number determination has the possibility of general application.

A SATISFACTORY and practical method for determining the iodine number of tall oil has become increasingly desirable. This material, derived from pine wood and consisting of approximately equal amounts of fatty and resin acids plus 6 to 10% of unsaponifiable matter, is a by-product of the alkaline sulfate paper pulp process.

Chapman, Hastings, and Pollak (*5*) recently reviewed this general subject and presented data on the application of the Wijs iodine method to tall oil. Their studies of the effects of temperature, excess iodine, and absorption time on the reaction showed that the resulting iodine values were markedly affected by these variables. Boesecken and Gelber (*4*) claimed that the Wijs method was not satisfactory for conjugated systems and that sulfur compounds interfered with the iodine absorption. Kubelka, Wagner, and Zuravlev (*14*) tried several methods for determining the iodine number of nonfatty materials of high molecular weight, such as turpentine and rosin oil. These methods were all very sensitive to time of reaction and sample weight. Forbes and Neville (*9*) obtained similar results with the Wijs method on conjugated drying oils. They suggested the use of the Wijs determination as a method of qualitatively indicating the presence of conjugated double bonds because of its extreme sensitivity to sample weight in this case. Dittmer (*8*) reported difficulties in iodine number determinations of tall oil and noted that the results showed deviations from the iodine values of common fatty acids, which, he explained, were caused by a polymerization phenomenon. An exhaustive discussion of the variables encountered in iodine number determinations is presented in a monograph by Margosches (*15*).

From the wide variety of iodine value ranging from 100–210 reported for tall oil by many investigators (*5*) and the experience of the authors with the Wijs method (which is in substantial agreement with that of Chapman, Hastings, and Pollak), it was obvious that no satisfactory and practical iodine number method for tall oil was available. Most of the compounds present in tall oil are highly unsaturated and are thought to be conjugated to an appreciable extent. They do not respond easily to ordinary methods of halogen addition.

Von Mikusch and Frazier (*26*, *27*) recently recommended the use of Hanus' solution, in which the concentration of iodine bromide is about doubled, for determining the total unsaturation of oils and fatty acids containing conjugated double bonds. Using 1-hour absorption at 20° C. and 500 to 800% excess reagent they found the iodine value of distilled tall oil to be about 204. This procedure, applied to crude tall oil, has not been tried by the authors, since apparently consistent results were obtained by the method described in this paper.

The advantages of using a more active halogen as the addition reagent and the possible use of a catalyst to promote the reaction were immediately apparent. Rosenmund, Kuhnhenn, Rosenberg-Gruszynski, and Rosetti (*22*) developed a bromine addition method using pyridine sulfate dibromide ($C_5H_5N.H_2SO_4.Br_2$) in glacial acetic acid solution approximately 0.1 *N* with respect to bromine. Except for the use of this reagent, the general proce-

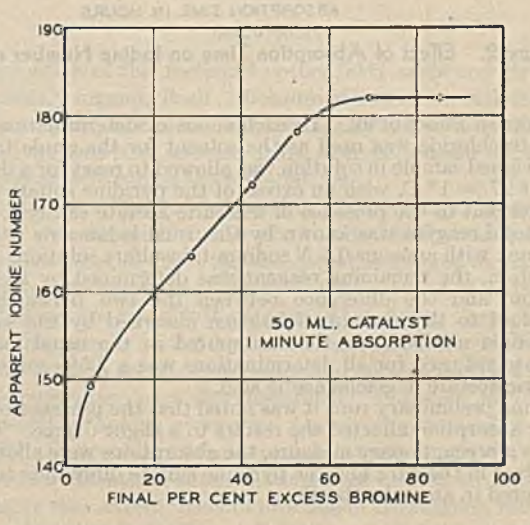

Figure 1. Effect of Excess Bromine on Iodine Number of Crude Tall Oil for Short Absorption Period

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dure is essentially the same as that of the Wijs method. The Rosenmund-Kuhnhenn method applied to oleic acid and cholesterol was tested and discussed by Yasuda (*29*), Dam (*6, 7*), and Page and Rudy (*18*). After comparative tests with various reagents Govindarajan (*10*) concluded that the Rosenmund-Kuhnhenn method was the most satisfactory for determining the iodine numbers of linseed, sunflower, and croton oils.

Rosenmund and Kuhnhenn also discussed further applications of their method (*21*) and the chemistry of pyridine sulfate dibromide (*20*). They showed that the compounds of bromine with pyridine or quinoline are active bromine addition agents and that they do not participate in the secondary reactions of substitution or oxidation. The solution is very stable over a long period of time and is much easier to prepare than the Hanus, Hübl, and Wijs solutions.

The use of a catalyst to promote absorption has been reported by several investigators. Hübl (*12*) suggested using mercuric chloride with an iodine-alcohol solution and Wijs (*28*) also reported his investigations in this connection. More recently Scotti (*24*) proposed the use of mercuric acetate, dissolved in glacial acetic acid, with an iodine-benzene reagent and claimed accelerated absorption by this method. Hoffman and Green (*11*) proved the superiority of mercuric acetate as a catalyst but preferred to use it with the Wijs solution. Mercuric acetate has also been tested with an elemental bromine–acetic acid reagent by Jasperson (*13*) and with the Hanus and Wijs reagents by Norris and Buswell (*17*).

DEVELOPMENT OF METHOD

In addition to the official Wijs iodine number procedure (*1, 2, 3*), the methods involving the use of mercuric acetate catalyst with the Wijs solution (*11*) and of pyridine sulfate dibromide reagent without a catalyst (*22*) were tried on crude tall oil. In each case, these procedures were unsatisfactory because of the extreme sensitivity of the results to the amount of excess reagent and the time of absorption.

Preliminary tests, in which mercuric acetate catalyst was used with the pyridine sulfate dibromide reagent, gave greatly improved results, however, and the most favorable conditions for carrying out the reaction were then determined.

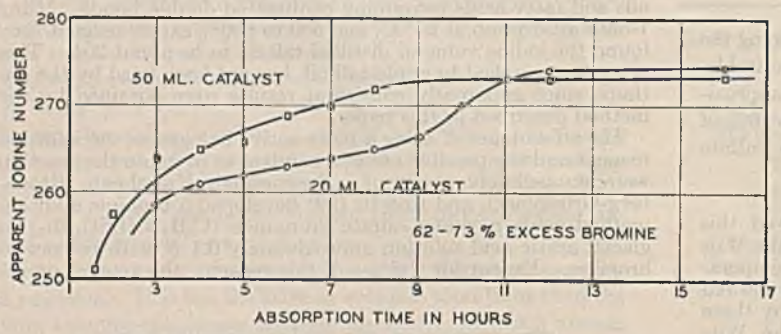

Figure 2. Effect of Absorption Time on Iodine Number of Crude Tall Oil

General Procedure. In each series of determinations carbon tetrachloride was used as the solvent for the crude tall oil. The weighed sample in solution was allowed to react for a definite time at 27 ± 1° C. with an excess of the pyridine sulfate dibromide reagent in the presence of mercuric acetate catalyst. The exact total reagent was known by the usual iodometric titration of a blank with aqueous 0.1 *N* sodium thiosulfate solution. After absorption, the remaining reagent was determined by a similar titration, and the difference between the two titrations was equivalent to the amount of halogen absorbed by the sample. The iodine number was then computed in the usual manner. The catalyst used for all determinations was a 2.5% solution of mercuric acetate in glacial acetic acid.

During preliminary runs it was noted that the presence of light during absorption affected the results to a slight degree. Therefore, as a precautionary measure, the absorptions were allowed to take place in the dark and the pyridine sulfate dibromide reagent was stored in an amber bottle.

For these reactants at a fixed temperature, the degree of absorption obtained is a function of the amount of catalyst, the length of the absorption period, and the amount of excess reagent For a fixed sample weight and a fixed original amount of reagent, the total excess reagent remaining after absorption will, of course, depend on the extent of the reaction. Hence, it is difficult to fix this variable absolutely, as, for example, when the effect of absorption time is being studied. The relatively slight variation, however, did not appear to affect the findings adversely.

Before studying the effect of absorption time it was desirable to obtain some idea of the amount of excess reagent necessary. A series of determinations was made with 50 ml. of catalyst solution for a short absorption period of one minute, using varying sample weights (Figure 1). Although the maximum iodine values obtained are low because of the short absorption period, the effect of per cent excess reagent disappears at values of about 60 to 70%.

Effect of Absorption Time. Using 62 to 73% excess reagent, two series of determinations were made (Figure 2). With 20 ml. of catalyst solution, absorption is complete in about 11 to 12 hours. When 50 ml. of catalyst solution are used, the reaction is complete in 9 to 10 hours. The initial absorption is comparatively rapid and from this fact one might deduce that the remaining slow absorption is by conjugated groups. A slight dilution effect is noticeable in Figure 2, where the larger volume of catalyst solution gives the lower iodine value. In preceding tests without the use of a catalyst, an absorption time of 75 hours was necessary to obtain an iodine value of 275 with no further absorption after 96 hours.

Effect of Excess Reagent. In order to determine if less excess reagent could be used with a longer absorption period, a series of analyses was made using 20 ml. of catalyst and 16 hours' reaction time. The results are plotted in Figure 3. The iodine value rises with increasing per cent of excess reagent and becomes constant at values above 50%. It can be concluded that 50% excess is the minimum amount of reagent with which reproducible results can be obtained under the above conditions.

SUGGESTED ANALYTICAL METHOD

Reagents. Aqueous starch indicator solution, prepared by adding 10 grams of soluble starch, made into a thin paste with cold water, to 500 ml. of boiling water. Add 10 grams of mercuric iodide to prevent bacterial action (*19*). The mercuric iodide is very sparingly soluble and will completely settle after standing about an hour.

Aqueous 15% potassium iodide solution. This solution will decompose and turn a pale yellow after standing for a long period. When necessary the color can be discharged by the addition of a drop or two of 0.1 *N* sodium thiosulfate.

Mercuric acetate in glacial acetic acid, 2.5% solution. Standardized aqueous 0.1 *N* sodium thiosulfate. Carbon tetrachloride.

Pyridine sulfate dibromide solution. Place 40 ml. of glacial acetic acid in each of three Pyrex Erlenmeyer flasks. To the first add slowly 16 ± 0.2 grams of pyridine with cooling. In the same manner add 20 ± 0.2 grams of concentrated sulfuric acid to the second flask. When cool, combine these solutions with further cooling, by adding the sulfuric acid–acetic acid mixture to the pyridine solution. To the third flask add carefully 16 ± 0.2 grams of bromine. Now add this solution to the mixture of the first two solutions and transfer to a 1-liter volumetric flask. Make up to the mark with glacial acetic acid and transfer to an amber or black-painted 2.5-liter glass-stoppered storage bottle. Add 1000 ml. of glacial acetic acid and mix the solution thoroughly.

Preparation and Weighing of Sample. Run all determinations in duplicate.

Transfer a sample of the approximate weight noted below from a beaker in which it can be warmed if necessary to a 500-ml. glass-stoppered bottle containing 25 ml. of carbon tetrachloride. To prevent possible loss of volatile halogen during the absorption period, use is recommended of the special iodine flasks with a lip around the top to provide a liquid seal of potassium iodide reagent. Weighing is accomplished by difference.

The following maximum sample weights are suggested for various oils in order that the proper amount of excess reagent will be present during absorption:

Approximate Iodine No.	Maximum Sample Weight, Grams
275	0.115 (about 3 drops)
100	0.250 (about 8–10 drops)
5–10	2–3 (about 100 drops)

For determinations on individual samples of highly unsaturated oils, it is desirable to make up 500 ml. of carbon tetrachloride solution in a volumetric flask and remove a 25-ml. aliquot portion for analysis which permits larger sample weight. This procedure is more accurate, because the error inherent in weighing by difference a very small sample of heated oil is reduced. However, this method requires large volumes of solvent and excess apparatus when a large number of routine determinations must be run.

Absorption and Titration. Run a blank with each series of determinations. To the weighed sample in carbon tetrachloride solution, add exactly 50 ml. of pyridine sulfate dibromide solution from a pipet (use water suction). Now, add 20 ml. of mercuric acetate catalyst. Allow the bottle to stand in a dark place for 16 hours at uniform room temperature. Add 20 ml. of 15% aqueous potassium iodide solution, allow to stand 1 minute, and then add 100 ml. of distilled water. Titrate in the usual manner with standardized 0.1 *N* sodium thiosulfate, using starch solution as the indicator. The end point should persist for 2 minutes.

Equivalent results can be obtained with 10 hours' absorption by using 50 ml. of mercuric acetate catalyst solution.

It is necessary to run blank and sample determinations at the same time and to use the same amount of catalyst in each case. A crystalline deposit of the catalyst is usually observed after the samples have stood for some time, probably because the solubility of mercuric acetate in acetic acid is depressed slightly when carbon tetrachloride is present. However, this phenomenon apparently has no adverse effect on the results.

Calculations for the iodine number are performed in the usual manner.

PRECISION OF THE METHOD

A series of twenty determinations using 20 ml. of catalyst and 16 hours' absorption time was run on individually weighed samples of crude tall oil taken from the same representative lot. The results were then statistically analyzed according to methods suggested by Scarborough (*23*) and Mellor (*16*). The most probable value of the iodine number of the particular sample was found to be 274.68 ± 2.09. The probable error of a single observation was 1.07%. The iodine number of crude tall oil will, of course, vary somewhat, depending upon the source of the oil. Values for eight different commercial samples of crude tall oil were found to vary between 237 and 287.

Results obtained by an experienced analyst on individually weighed duplicate samples of tall oil which are within 5 or 6 iodine number units of each other may be regarded as good checks. Undoubtedly, the chief error lies in the weighing, because the sample must necessarily be small for such a highly unsaturated substance. It is difficult to weigh warm oil samples by the difference method with good accuracy, and a direct method of weighing with the use of microbeakers should permit some improvement.

ABSENCE OF SECONDARY REACTIONS

If secondary substitution or oxidation reactions took place along with addition to the double bonds, hydrobromic acid would be present in the reaction mixture after absorption. A sample of crude tall oil, along with a blank, was allowed to stand 23 days in the presence of an excess of the pyridine sulfate dibromide reagent and 20 ml. of the mercuric acetate catalyst solution. Distilled water was added and the free bromine was then removed by successive extractions with carbon tetrachloride. Acidification of the water layer with sulfuric acid and subsequent oxidation with hydrogen peroxide gave a negative test for bromide ion, using the sensitive fluorescein method of detection (*25*). This result is evidence of the absence of a secondary substitution reaction and substantiates the claim of Rosenmund and Kuhnhenn (*20*) in this respect.

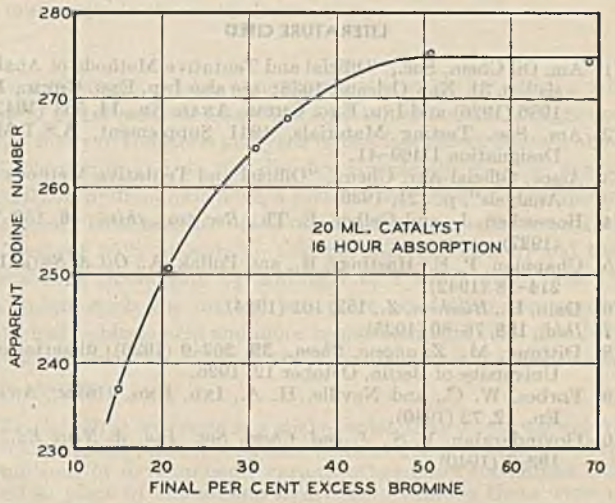

Figure 3. Effect of Excess Bromine on Iodine Number of Crude Tall Oil for Long Absorption Period

The reported iodine value does not necessarily represent with great accuracy the true total carbon-to-carbon unsaturation of crude tall oil. Reactions other than carbon-to-carbon addition or the secondary substitution, shown above to be absent, although improbable, may occur and cause the apparent iodine value to be higher than the true one for carbon-to-carbon unsaturation alone. However, the results obtained appear to be consistent, and the method proposed has distinct value for purposes of identification and analytical control. For example, the degree of hydrogenation of a partially hydrogenated pilot-plant sample of crude tall oil was computed from iodine numbers determined by this method. The result agreed almost exactly with that obtained by graphical integration of the differential rate curve, determined experimentally by observations of hydrogen pressure drop.

DISCUSSION

Application of this method to other fatty acids and glycerides immediately suggests itself. Because of its success with tall oil, it is especially recommended for other highly unsaturated and conjugated systems such as rosin acids, tung oil, linseed oil, natural resins, synthetic rubberlike materials, etc.

Although the effect of temperature on the rate of bromine absorption was not studied, this effect undoubtedly does exist. It is therefore necessary that the absorption be allowed to take place at constant temperature. When a large number of routine determinations are made, it is suggested that during the absorption period the sample bottles be placed on a rack, provided with a cover or hood to keep out the light, and partly immersed at constant temperature in a thermostatically controlled water bath. It is possible that an absorption temperature somewhat higher than 27° C. may shorten the required absorption period.

Further refinements of this method suggest themselves, such as increasing the concentration of bromine in the reagent, leading to the use of either less volume of reagent or a greater quantity of sodium thiosulfate solution for the titrations, the use of auto-

matic pipets and highly accurate thin-bore burets, and the development of a micromethod. The reader is referred to von Mikusch and Frazier (*26*) and Yasuda (*29*) for specific examples.

ACKNOWLEDGMENT

The authors are indebted to Harold G. Cassidy and Charles O. Edens, Department of Organic Chemistry, Yale University, for their suggestions in connection with the development of this method. This work was done under part of a fellowship grant sponsored by Dictaphone Corporation for the year 1942.

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An Improved Vacuum Distilling Head

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ONE of the most convenient setups for ordinary vacuum distillation consists of a Claissen flask in conjunction with a distilling flask or another Claissen flask as a condenser and receiver. This arrangement has several advantages. It may be set up and taken down rapidly; a good vacuum can be readily obtained; there is no appreciable holdup of the distillate between still and receiver; and, after the distillation, the distillate is in a still ready for distillation again if need be. On the other hand, the necessity of keeping on hand many different sizes of flasks is a serious disadvantage, since these flasks have little use except for distillations.

The apparatus in Figure 1 was designed to overcome this disadvantage, and also for convenience in setting up the still without sacrificing any of the advantages of the Claissen flask.

Openings 3, 4, and 8 are for capillary tube, thermometer, and vacuum, respectively. Standard-taper joints at 2 and 7 greatly facilitate change of flasks 1 and 5. Drip tip 6 helps prevent holdup in the delivery tube. Flask 5 is cooled with running water or a bath of ice or solid carbon dioxide. No other condenser is needed.

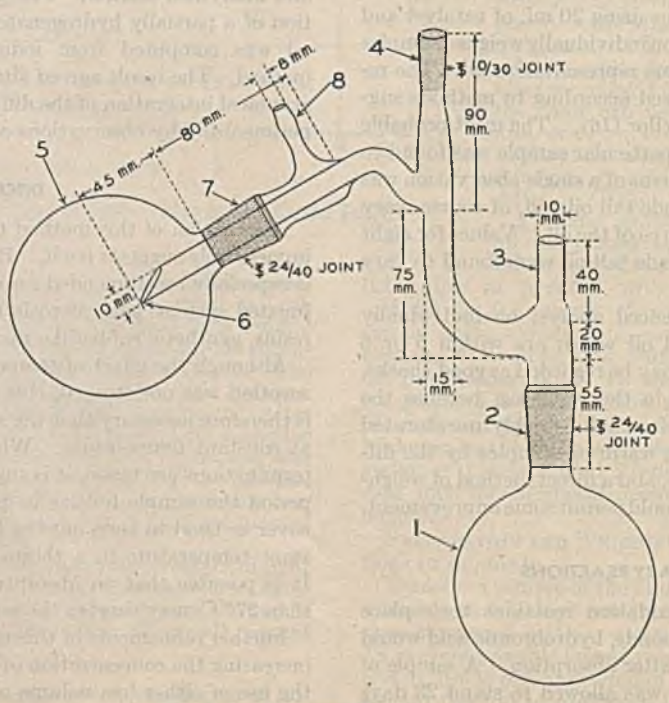

Figure 1. Vacuum Distilling Head

The flasks, when not in use for distilling, may be used for other purposes. The distilling head is simple to construct and takes very little space. All the advantages of the Claissen flasks are retained without the need of a large stock of flasks which have only one use. With this apparatus it is practical to use flasks of 50- to 2000-ml. capacity without altering the dimensions of the head. A number of laboratory supply houses make a standard-taper Claissen-type distilling head, but in order to use their apparatus for a vacuum distillation either an auxiliary condenser or a vacuum adapter is necessary, which increases the holdup and complexity of the apparatus.

Colorimetric Determination of Nickel in Steel

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The method of Murray and Ashley for the colorimetric determination of nickel in steel is outlined and criticized. Experimental data are presented on the stability under various conditions of the red color of oxidized nickel dimethylglyoxime. A modification of the method is described which gives a highly stable and readily reproducible red color, and is particularly suitable for routine work because of its rapidity and manipulative simplicity. Copper and cobalt interfere only slightly; the other elements ordinarily found in steel do not interfere. The accuracy of the method is comparable to that of routine gravimetric procedures.

MURRAY and Ashley (*1*) have presented a method for developing quantitatively the red color of oxidized nickel dimethylglyoxime.

The sample is dissolved in dilute nitric acid (in the case of difficultly soluble chromium-nickel steels, a mixture of nitric and hydrochloric acids is used). To an aliquot of suitable size the following additions are made: citric acid to prevent iron precipitation at the final pH, bromine water to oxidize nickel, ammonium hydroxide in sufficient quantity to bring the pH to 8–9, and dimethylglyoxime in the form of 1% solution in alcohol to develop the red nickel color. The solution is diluted to known volume and the color is compared. A wave length that has been recommended for spectrophotometric reading is 530 mμ.

Although the method of Murray and Ashley has been used with some success in this laboratory and elsewhere, it has certain undesirable characteristics. The color developed in the nickel solution shows continuous change from the instant of addition of dimethylglyoxime, tending for the first few minutes to become more intense, then to fade. Murray and Ashley note the phenomenon of the color intensity change at 530 mμ with time, and present a set of curves illustrating its characteristics. At no time is there a period of constant transmittance in the transmittance *vs.* time curve of sufficient duration to give a time margin for truly reproducible reading.

Both the slope and the time of the minimum of the transmittance *vs.* time curve appear to be changed by changes in the nickel concentration of the solution used for making the curve. Small differences in pH at the time of adding dimethylglyoxime to otherwise similar solutions were found to have pronounced effects on the shape of the transmittance *vs.* time curve, altering both the slope and the position of the minimum to a marked degree.

If 80% of the recommended amount of ammonium hydroxide is used, no color at all develops (pH 6.8). (All pH measurements were made with a Leeds & Northrup potentiometric pH meter using a standard glass electrode and a calomel–saturated potassium chloride reference electrode.) With 90% of the amount required (pH 8.1), the rate of color development is slow and rate of fading is high. If the concentration of ammonium hydroxide is high, the rate of color development is high and the rate of fading slow, solutions retaining substantially all their color for several hours. If as much as four times the recommended amount of ammonium hydroxide is used, the color is completely developed in about 30 seconds. A second effect enters, however—a rapidly increasing interference of the iron in the solution. Ultimately iron hydroxide is precipitated under these conditions. Critical examination of the pH range within which the Murray-Ashley method is workable shows it to be less than 1 pH unit, pH 8.2 to 8.7. It was found that the amounts of tartaric acid, bromine water, and dimethylglyoxime solution were not critical above a minimum value if sufficient extra ammonium hydroxide was added to neutralize increases in the acid content.

It was concluded that the unmodified method of Murray and Ashley, while suitable for many purposes, is not sufficiently reproducible (at least without elaborate precautions) to meet the needs of this laboratory. It does, however, have the virtue of being rapid. It was in an effort to retain its speed while improving its accuracy that this research was undertaken. The original method recommends dissolving the sample in 1 to 1 nitric acid, except difficultly soluble chromium-nickel steels for which a mixture of equal parts of nitric and hydrochloric acids is suggested. Since many of the steels analyzed in this laboratory contain about 1% chromium and do not dissolve completely in nitric acid or rapidly in hydrochloric acid, a method was tried of decomposing first in an "acid mixture" (133 ml. of 1.82 sp. gr. sulfuric acid and 167 ml. of 85% phosphoric acid per liter of solution) and then completing dissolution by adding 1 to 1 nitric acid. For 1% chromium steels the method was more rapid than decomposing with hydrochloric acid and more nearly complete than dissolving in nitric acid. No effect on the development of the nickel color was observed.

In the effort to produce a nickel color that would be quickly formed, stable, and reproducible without elaborate control of the conditions of development, various other basic substances were tried in place of ammonium hydroxide. Among them were sodium carbonate, sodium tetraborate, sodium orthophosphate, potassium pyrophosphate, and sodium hydroxide, all in the concentrations required to produce the proper pH range for color development. None proved satisfactory. Other oxidizing agents such as sodium perborate, potassium chlorate, potassium iodate, and hydrogen peroxide were tried in place of the bromine. Only ammonium peroxydisulfate in the presence of silver ion showed promise, but the desired degree of color stability was not achieved with it. The effects of solution temperature on color development were studied, but no worthwhile modification utilizing temperature control was found. Color development at high ammonium hydroxide concentration followed by reduction of the solution pH to 8.5 to prevent iron precipitation proved unworkable.

It was realized at this point that tartaric acid will hold iron in solution at a considerably higher pH than will citric acid. Accordingly, the citric acid was replaced by tartaric. Upon experiment it was discovered that, while iron will develop an interfering color in tartrate solutions made basic with ammonium hydroxide nearly as quickly as in citrate solutions under the same conditions, the interference and any precipitate that may form can be cleared up rapidly by increasing the pH still further with sodium hydroxide. This is not true of citrate solutions. While the nickel color develops slowly and incompletely in very dilute sodium hydroxide solutions of pH 8 to 9 and not at all in more concentrated solutions, the color, once developed, remains stable over long periods of time even in rather strongly basic sodium hydroxide solutions. In view of these facts, the following approach was tried:

The sample was treated with tartaric acid and bromine and made strongly ammoniacal. Then the dimethylglyoxime solution was added. Under these conditions the color developed rapidly. After 1 minute, sodium hydroxide was added. After 2 to 3 minutes the increase in iron interference which had taken place in ammoniacal solution cleared up completely and the color became stable. No further change in color and consequently in transmittance at 530 mμ was observed. The reading at 24 hours was identical with the reading 5 minutes after adding dimethylglyoxime.

PROCEDURE

Since these results seemed very encouraging, a procedure was devised to make use of them and was used in all the studies hereafter reported. High-purity reagents must be used. Particular

attention should be paid to the suitability of the tartaric acid and of the dimethylglyoxime.

Decompose a 0.25-gram sample in 20 ml. of an acid mixture (133 ml. of 1.82 sp. gr. sulfuric acid and 167 ml. of 85% orthophosphoric acid per liter of solution). Steels containing little chromium may be dissolved directly in 8 *N* nitric acid. Stainless-type steels may require the use of hydrochloric acid. Cautiously add 10 ml. of 8 *N* nitric acid and boil to expel oxides of nitrogen. Transfer the solution to a volumetric flask of suitable size and dilute to the mark. Transfer by pipet to a 100-ml. volumetric flask an aliquot of the diluted solution containing between 0.05 and 0.3 mg. of nickel. Add to it, mixing after each addition, 5 ml. of a 20% tartaric acid solution, 5 ml. of saturated bromine water, 10 ml. of 0.90 sp. gr. ammonium hydroxide, and 5 ml. of a 1% solution of dimethylglyoxime in methyl alcohol. (Occasional difficulties in development of color and in fading upon addition of sodium hydroxide have been traced to impure or partially decomposed tartaric acid and dimethylglyoxime. C.P. reagents are not uniformly satisfactory in this respect. Impure tartaric acid interferes with development of color upon addition of dimethylglyoxime, in extreme cases preventing any color formation at all. Impure dimethylglyoxime results in a fading of the color upon addition of sodium hydroxide. The fading may take place very rapidly or slowly, depending on the degree of impurity. Both difficulties can be overcome by special treatment. Addition of a second 5-ml. portion of bromine water after introduction of the dimethylglyoxime ensures complete color development. Difficulties with the dimethylglyoxime reagent can be overcome by acidifying the alcohol solution with dilute sulfuric acid and adding enough bromine water to color it yellow. This should be done 15 to 30 minutes before it is used. Occasional small further additions of bromine water are necessary to keep the solution yellow. The treated reagent is usable for only a few hours.)

After 1 minute add 10 ml. of 6 *N* sodium hydroxide solution and dilute to the mark. After 5 minutes, transfer the solution to the optical cell and compare the transmittance at 530 mμ with that of pure water.

Two transmittance *vs.* wave-length curves were prepared, one from National Bureau of Standards Bessemer steel 10d containing substantially no nickel, and one from a nickel solution made from C.P. nickel nitrate. They are shown in Figure 1 as read on a Coleman Universal spectrophotometer. A Beckman spectrophotometer, with a much narrower wave band than the Coleman, gave a similar curve for the nickel solution; the positions of the maxima and minima were identical. Appreciable interference of the blank (Bessemer steel), however, did not occur on the Beckman instrument until wave lengths as short as 470 mμ were reached.

From a study of these curves it was decided that the most satisfactory wave length for reading the transmittance of the nickel color in steel on a Coleman Universal spectrophotometer, which has a wave band width of 35 mμ, is 530 mμ, the wave length originally suggested for the method of Murray and Ashley. This selection was made on a basis of minimum interference of the blank and maximum interference of the nickel color. For instruments with a wave band much narrower than 35 mμ, such as the Beckman photoelectric spectrophotometer, a lower setting such as 480 or 490 mμ seems to be preferable, since the lower value is closer to a minimum of the nickel curve and since iron interference is small in this range with such an instrument. Presumably, it would be possible to use for both spectrophotometers a wave-length setting at the 470 mμ minimum of the nickel curve, if a blank prepared from a steel free of nickel were used as the reference solution, thus canceling the effect of iron interference.

For colorimeters using filters one probably would do best to select a filter with a rather sharp cutoff at about 450 mμ, passing no light of shorter wave length. This selection is indicated to eliminate interference due to the color of the iron present. Increased sensitivity can be obtained by further restriction of the wave length of light used for comparison to the range of maximum interference of the nickel color.

Samples were taken from a series of National Bureau of Standards nickel-containing steels and mixtures of these steels to cover in small steps a series of nickel percentages from 0.002 to 5.12.

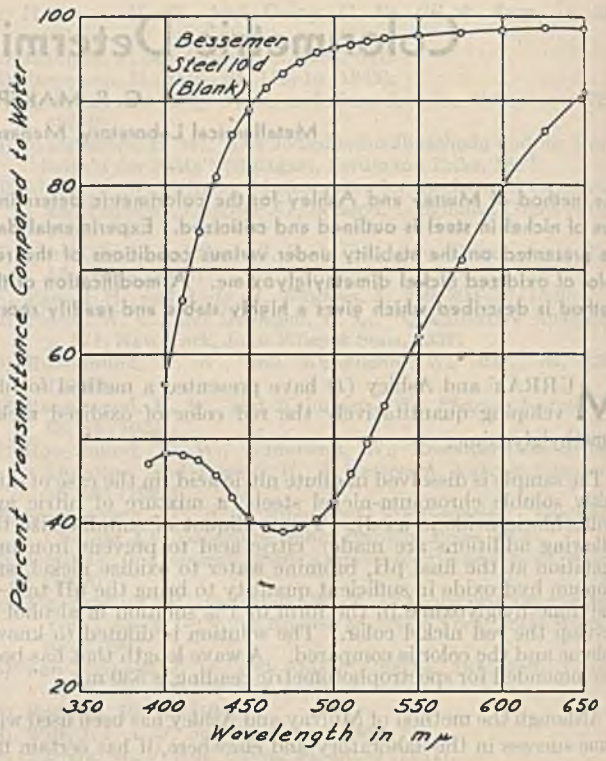

Figure 1. Per Cent Transmittance *vs.* Wave Length

Curves for Bessemer steel blank and C.P. nickel nitrate solution as read on Coleman Universal spectrophotometer (35 mμ band width)

They were prepared according to the method previously outlined, and the points so obtained were plotted on semilog paper with the per cent transmittance of the sample compared with water at 530 mμ as the ordinate and the per cent of nickel in the steel as the abscissa. In this fashion three transmittance *vs.* concentration curves were obtained, for 0 to 1%, 1 to 2.5%, and 2.5 to 5.5% nickel steels. A total of 27 concentrations was used in obtaining the three curves.

The color was found to follow the Beer-Lambert law very exactly when read at 530 mμ; therefore the transmittance *vs.* concentration curves, when plotted as described, were straight lines over the entire range utilized.

No one of the 27 points determined from the National Bureau of Standards samples deviated from the transmittance *vs.* concentration curves plotted from them by more than 2% of the total nickel present in the steel in the case of steels containing more than 1% nickel. No one of the points deviated from the transmittance *vs.* concentration curve by more than 0.02% nickel (2 "points") based on the total analysis of the steel in the case of steels containing less than 1% nickel. These points were established by single determinations, not by averages of groups of determinations. The differences between the high and the low values on the National Bureau of Standards reports sent with each steel are as great as 1.7% of the total nickel present in the steel in the case of steels containing more than 1% nickel. Differences of as much as 0.021% of nickel (2 "points") based on the total analysis of the steel are listed in the case of steels containing less than 1% nickel.

Reproducibility was checked by making five complete determinations by the authors' method on National Bureau of Standards nickel steel 33b containing 3.48% nickel. Using the transmittance *vs.* concentration curve established for steels containing 2.5 to 5.5% nickel, the average value of the five determinations was 3.48% nickel. The maximum deviation from the average value was −0.05% nickel. High and low values on the National Bureau of Standards report are 3.51 and 3.46% nickel, respectively. The method appears to be capable of the high order of reproducibility necessary for precise steel analysis.

SENSITIVITY

Tests were made to determine the sensitivity of the method to small variations in procedure. It was found that the tartaric acid, bromine water, and dimethylglyoxime added could be decreased 25% or increased 100% without affecting the results. The ammonium hydroxide may be decreased 20% or increased 50% without effect. Approximately the same range of values holds for the sodium hydroxide. It was determined that the time elapsed between adding the dimethylglyoxime and the sodium hydroxide is not critical so long as it exceeds 1 minute—i.e., color development is complete in less than 1 minute.

Three identical samples equivalent to a standard steel containing 0.60% nickel were prepared, using for each sample 0.125 gram of National Bureau of Standards Bessemer steel 10d and 0.125 gram of nickel-chromium steel 32c. To sample 1 the sodium hydroxide, which arrests color development as well as preventing iron precipitation, was added 30 seconds after adding dimethylglyoxime; to sample 2, 5 minutes after adding the dimethylglyoxime; and to sample 3, 10 minutes after adding the dimethylglyoxime. In each case the solution was diluted to the volumetric mark and mixed immediately after adding the sodium hydroxide. Transmittance readings in each case were taken 10 minutes after adding the sodium hydroxide. The results, expressed in terms of the analysis of the steel as read from the 0 to 1% nickel curve, averaged 0.599% nickel with a maximum deviation from the average value of 0.005% nickel.

INTERFERENCES

The method was tested for interference by copper, cobalt, tungsten, molybdenum, chromium, and vanadium. The small amounts of copper (less than 0.2%) present in the usual steels did not interfere. Copper, when present to the extent of 0.50% in the steel, caused a positive nickel error of 0.02%. Cobalt, when added to the sample equivalent to 2.5% in the steel, caused a positive error of 0.03% nickel. Both elements were tested for interference on a sample of steel containing 0.60% nickel. The interference of the other elements mentioned was determined by using the method without modification of any sort to analyze for nickel in two Bureau of Standards tool steel samples, 50a and 132.

Table I. Analysis of Steels

	Sample 50a		Sample 132	
	Bureau of Standards %	Authors' method %	Bureau of Standards %	Authors' method %
Tungsten	18.25	..	6.29	..
Chromium	3.52	..	4.09	..
Vanadium	0.970	..	1.64	..
Nickel	0.045	0.07	0.095	0.13
Molybdenum	..	..	7.08	..

In view of the extreme conditions in these two analyses and the small error in nickel in each case, it was concluded that these elements do not interfere with the determination to any appreciable extent. In the analysis of ordinary chromium-nickel stainless steels, no interference by chromium was observed.

ADVANTAGES OF METHOD

The most important advantages of the method are its rapidity and its freedom from manipulations requiring exceptional precautions or a high degree of analytical skill. It is well suited for routine use. When a group of five samples was analyzed by a worker only recently familiar with the method, the total elapsed time, exclusive of weighing, was 1 hour and 10 minutes (results reported above in paragraph on reproducibility). If a large number of samples is run at one time, one thoroughly familiar with the method can reduce the time required per sample to about 8 minutes.

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Identification of Nornicotine in Tobacco

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THE rather common occurrence of nornicotine in tobacco (*3*, *4*, *6*, *7*) indicates the need of a reliable qualitative means of identifying this alkaloid when present with nicotine.

Shmuk (*8*) identified nornicotine in such a mixture after extracting the ether-soluble material from alkalized plant material, by forming the alkaloid picrates, recrystallizing from hot water, and methylating the mixture of picrates. He attributed the resulting elevation in melting point of the picrate to the conversion of nornicotine to nicotine.

This procedure has several serious drawbacks. The ether extract of the alkalized plant material will contain pyridine and other amines, not all of which are removed by "blowing". These compounds as well as the alkaloids form picrates. The recrystallizations that occur before and during methylation will tend to remove these amine picrates but at the same time to eliminate the picrate of the minor alkaloid. If the minor alkaloid is present in small amount, it may be lost in the recrystallization of the original picrate. Since the mixed picrates before methylation have a melting point lower than that of nicotine picrate, and, being solid, require the addition of liquid for the methylation, it is possible for a fractional crystallization to occur with the formation of new picrate crystals richer in nicotine content and consequently having a higher melting point. Such a rise in melting point could easily be misinterpreted as being due to methylation of nornicotine, while in fact the alkaloid picrate originally present may have been simply rendered richer in nicotine by recrystallization. Such a recrystallization proves neither the presence nor the absence of nornicotine.

The proposed method differs from that of Shmuk in the isolation of the alkaloids and in the position of the methylation step. The steam-volatile tobacco alkaloids are separated from all other amines and methylated before the picrate is formed. The melting points of the picrates formed are compared before and after methylation. Since they are not recrystallized no fractional crystallization can occur.

Markwood (*5*) introduced methylation as a step in the determination of nornicotine, but since his method did not depend on melting points, he failed to report the melting point of methylated nornicotine picrate as an indication of the formation of nicotine.

The method presented here is based on the quantitative methylation of the nornicotine and also on the elevation of the melting point of the alkaloid picrate. Consequently, the methylation of nornicotine to nicotine was investigated. The nornicotine used in preparing the standard solution was identical with that used by Markwood (*7*), had been isolated from Robinson's Medium Broadleaf, a strain of Maryland tobacco, and formed a picrate melting at 190–191° C. The absence of nicotine was established by finding no alkaloid in the distillate after treating a sample with nitrous acid, making the aqueous solution just basic to phenolphthalein, and steam-distilling.

Table I. Effect of Methylation of Nornicotine on Picrate Melting Point

Sample	Nornicotine in Alkaloid Mixture	Picrate Melting Point[a] Original	Methylated	Mixed
	%	° C.	° C.	° C.
Nicotine	...	221–223	.....	.....
Nornicotine	100	187–190	221–223	220–223
Mixtures				
1	94.8	168–180	220–223	221–223
2	89.4	180–184	223–224	221–223
3	84.3	175–183	222–223	221–223
4	79.2	178–183	222–223	220–221
5	22.9	205–214	222–224	221–224
6	19.2	210–218	222–224	222–224
7	13.7	213–220	222–224	222–224
8	4.6	215–223	222–224	222–224

[a] Not recrystallized. Melting points corrected and rounded off to whole numbers.

METHYLATION OF NORNICOTINE

Ten milliliters of the nornicotine solution containing 23.2 mg. of the alkaloid were treated with formic acid and formaldehyde according to Markwood's method (*5*), made alkaline to phenolphthalein, and steam-distilled. No alkaloid was detected in the distillation residue.

The alkaloid in the distillate was precipitated as a picrate, the melting point of which, when not recrystallized, was 221–223° C. A mixed melting point with nicotine picrate prepared from a sample of pure nicotine showed no depression. The pure nicotine had been treated with nitrous acid for the removal of nornicotine. The absence of alkaloid in the distillation residue and the melting point of the picrate of the alkaloid in the final distillate prove the quantitative methylation of nornicotine to nicotine by means of formaldehyde and formic acid. A sharp melting point is not to be expected after methylation when no recrystallization has been made. In obtaining the melting points, consideration must be given to the spread, although the upper limit is the most easily observed temperature. The entire spread must be considered as the melting point. Values obtained with known mixtures of nicotine and nornicotine are shown in Table I.

PROCEDURE

A 1-gram sample of tobacco, 10 ml. of sodium hydroxide (30% by weight), and 10 grams of sodium chloride are steam-distilled into 3 ml. of dilute hydrochloric acid (1 + 4) until a fresh portion of distillate gives no opalescence when a few milliliters are tested with silicotungstic acid solution. The volume of distillate is about 100 ml. The steam-volatile alkaloids are separated from other picrate-forming materials by precipitation by silicotungstic acid solution (12%) according to A.O.A.C. procedure (*1*), filtered on a small, hardened filter paper in a Hirsch funnel, and washed with water containing 1 ml. of concentrated hydrochloric acid per liter. The alkaloid in this residue is steam-distilled from sodium hydroxide and sodium chloride into hydrochloric acid, as above. The distillate is concentrated to about 10 ml. and divided into two approximately equal portions. One portion is methylated according to Markwood's method by adding 2 drops of formic acid and 5 ml. of formaldehyde (37%) and refluxing for 15 minutes. It is then cooled and neutralized, and 25 ml. of saturated aqueous picric acid solution are added. This solution is next concentrated by boiling to about 30 ml. and allowed to cool slowly. Twenty-five milliliters of the picric acid solution are added to the neutralized unmethylated portion, warmed to dissolve the precipitate, cooled slowly. The alkaloid picrates are filtered off and washed, once with dilute picric acid solution and once with water. The melting points of the picrates of the unmethylated distillate, the methylated portion, and the methylated portion mixed with nicotine picrate are compared for an elevation of temperature due to methylation. The nicotine picrate should be prepared from pure nicotine and washed in the same manner as the unmethylated portion.

If the melting point of the methylated portion is higher than that of the untreated portion and is comparable with that of the nicotine picrate, and no depression occurs in the mixed melting point, nornicotine is confirmed. Since nornicotine is the only alkaloid that can be methylated to form nicotine and any derivative of other alkaloids would affect the mixed melting point, it is evident that nornicotine is the only steam-volatile alkaloid aside from nicotine which is present in the tobacco tested. Table II shows the effect of methylation.

Nornicotine in tobacco, tobacco mixtures, and nicotine preparations may be identified in the same manner, although smaller samples should be used when the alkaloid content is high.

Although this method appears to be lengthy, at present it offers the best chemical means for the identification of nornicotine, can be conducted in any laboratory, and does not require expensive and specialized equipment, such as a polarimeter, which is now either not available or difficult to obtain. It has the following advantages over the method proposed by Shmuk: (1) When the picrates are formed, only the steam-volatile alkaloids rather than all the ether-extractable materials are present; (2) since the mixed picrate is recrystallized only in the mother liquor instead of being recrystallized from hot water and again in the methylation process, the possibility of loss of the minor alkaloid is eliminated; and (3) the steam-distillation requires less time than an ether extraction. An improved steam-distillation apparatus (*2*) was used, but any steam-distillation apparatus may be conveniently used although the time may be longer and the volume of the distillate larger. When nicotine and nornicotine are determined (*3*), aliquants of the first distillate may be used to confirm the presence of nornicotine.

Table II. Effect of Methylation of Tobacco Alkaloids on Picrate Melting Point

Sample	Analysis Nicotine	Analysis Nornicotine	Nornicotine in Total Steam-Volatile Alkaloid	Picrate Melting Point[a] Original	Methylated	Mixed
	%	%	%	° C.	° C.	° C.
Cash (flue-cured type)	0.70	2.40	77.4	182–185	221–223	220–222
Maryland Medium Broadleaf (Robinson)	0.98	2.18	68.8	180–194	222–223	219–223
Burley, Halley	1.23	1.41	53.4	196–206	220–223	221–224
Maryland, Md.-Conn. Broadleaf	2.22	0.49	18.1	203–219	222–223	222–223
Xanthi (Turkish)[b]	6.59	1.28	16.3	214–223	221–224	221–224

[a] Not recrystallized. Melting points corrected and rounded off to whole numbers.
[b] American grown.

SUMMARY

Nornicotine may be identified in tobacco, insecticidal tobacco preparations, and nicotine preparations by comparing the melting point of the mixed picrates of the steam-volatile alkaloids with the picrate melting point of a methylated sample thereof. Methylation of the nornicotine gives nicotine; consequently, the picrate of the methylated alkaloids will melt at the same point as nicotine picrate and no depression of melting point will occur in a mixed-melting point determination with nicotine picrate in those cases where steam-volatile alkaloids other than nicotine and nornicotine are substantially absent.

Nornicotine was confirmed in tobacco samples by this method.

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Colorimetric Determination of Phosphorus as Molybdivanadophosphoric Acid

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A spectrophotometric study of the molybdivanadophosphoric acid method for the determination of phosphorus justifies its general recommendation for measuring this element colorimetrically. The experimental work covered the effects of the following variables: acidity, reagent and phosphorus concentrations, temperature, order of adding reagents, stability, and some 60 diverse ions. As one result, an improved method is proposed for applying the method to determining phosphorus in plain carbon and low-alloy steels.

A YELLOW hue forms on adding an excess of a molybdate solution to an acidified solution mixture of a vanadate and an orthophosphate. Presumably the colored component formed is molybdivanadophosphoric acid.

Mission (*6*) first proposed using this reaction as a basis for a colorimetric method for determining phosphorus in steel. Later Getzov (*1*), Schröder (*8*), and Murray and Ashley (*7*) used it for steels, and Willard and Center (*10*) modified it for iron ores. Recently Koenig and Johnson (*4*) applied it to biological materials.

The general objective of the present work was to extend our knowledge of the analytical possibilities of this mixed heteropoly acid, since it is one of the few known examples of such compounds having direct value in colorimetry. More specifically, it seemed desirable to determine the general applicability of the procedure, including its merits relative to other available colorimetric methods for phosphorus, and to examine more critically its application to the determination of phosphorus in steel.

GENERAL EXPERIMENTAL WORK

APPARATUS AND SOLUTIONS. Transmittancy measurements were made in 1.000- or 5.00-cm. cells with a General Electric recording spectrophotometer adjusted for a spectral band width of 10 mμ. If necessary to correct for a pale color in the reagents, a compensating blank was used in the reference beam of the spectrophotometer. Otherwise distilled water was used.

A standard solution containing 0.1 mg. of phosphorus per ml. was prepared by dissolving twice recrystallized potassium hydrogen phosphate in water. A 5% solution of ammonium molybdate was prepared by dissolving the salt, $(NH_4)_7Mo_7O_{24}.4H_2O$, in warm water (50° C.). The vanadate solution was made by dissolving 2.5 grams of ammonium vanadate, NH_4VO_3, in 500 ml. of boiling water, cooling the solution somewhat, adding 20 ml. of concentrated nitric acid, and diluting to 1 liter after allowing the mixture to cool to room temperature. To determine the effect of diverse ions, nitrate, sulfate, or acetate salts were used for the cations, and sodium, potassium, or ammonium salts for the anions. The various acids were analytical grade reagents.

COLOR REACTION. Although Mission (*6*) formulated the ammonium salt of the heteropoly complex as $(NH_4)_3PO_4.NH_4VO_3.16MoO_3$, the exact nature of the compound is not clear. It cannot readily be fitted into either the Rosenheim or the Keggin formulas for heteropoly compounds. Presumably the acid is formed by substitution of both molybdenum and vanadium oxide radicals for oxygen in the phosphate radical to give a mixed heteropoly compound. If such is the case, the ratio of vanadium to phosphorus would have to be at least 2 to 1, and the ratio of molybdenum to phosphorus not greater than 10 to 1.

Application of the method of Vosburgh and Cooper (*9*) to determine these ratios was tried. Although previously this process has been used on relatively simple systems, it seemed that the ratio of vanadium to phosphorus might be determined by holding the molybdate and acid concentration constant. However, the results indicated that the ratio of vanadium to phosphorus is 1 to 1. Attempts to determine the ratio of molybdenum to vanadium plus phosphorus failed, probably because most color reactions involving a heteropoly molybdate require a large excess of molybdate for color development. If one may reason from the results obtained, it appears that the compound is not of the type represented by the Rosenheim formulation $H_7P(Mo_2O_7)_n$-$(V_2O_6)_{6-n}$. Although the constitution of molybdivanadophosphoric acid remains uncertain, fortunately the usefulness of the colorimetric procedure is unaffected.

EFFECT OF VARIABLES ON THE COLOR DEVELOPMENT. To study the effect of variables on the color development, the following experimental procedure was used:

A definite volume of phosphate solution, usually 5 ml., was placed in a 50-ml. volumetric flask, together with enough water to bring the volume of solution to about 20 ml. Five milliliters each of nitric acid (1 to 2), 0.25% ammonium vanadate solution, and 5% ammonium molybdate solution were added in order. Any precipitate formed was dissolved by mixing. The solution was then diluted to the mark with water and mixed, and the transmittancy curve determined.

Acid Concentration. In all previous work, except that of Willard and Center (*10*), nitric acid was used. If it is employed, there must be enough present to prevent the appearance of an orange-yellow color which forms in neutral or slightly acidic solutions. If the acidity exceeds 0.2 *N*, this color does not appear. Additional acid, up to 1.6 *N*, has no effect on the color, except that it develops more slowly at the higher acidities. At acidities above 1.6 *N* the color forms so slowly that a considerable negative error may arise. The optimum acidity of about 0.5 *N* is readily secured by using 5 ml. of nitric acid (1 to 2) per 50 ml. of final volume.

Sulfuric, perchloric, and hydrochloric acids behave much like nitric acid. If the acidity with any of them is less than 0.2 *N*, the orange-yellow hue develops in the blank. The desired color appears only slowly with 1 *N* hydrochloric acid, but development is complete within 5 minutes. Solutions 0.7 *N* in sulfuric acid, or 0.9 *N* in perchloric acid, behave similarly. At normalities of 1.4 and 1.7, respectively, full color is not developed in 5 minutes.

Vanadate Concentration. An excess of vanadate must be present for complete color development. Beyond this amount, additional reagent has no effect except for the slight color of the vanadate solution. For the present working conditions, 10 ml. (±1) of 0.25% reagent were satisfactory.

Molybdate Concentration. As in most procedures involving heteropoly compounds, excess ammonium molybdate must be present for complete color development. Beyond this amount, more reagent has no effect on the intensity of the color. Although prior recommendations specify a 10% solution, this has been changed to 5% because the solution is more easily prepared, the extra reagent is unnecessary, and the smaller concentration avoids the formation of the precipitate which may appear with use of the more concentrated solution.

Order of Adding Reagents. The reagents should be added in the order mentioned. If the acid follows the vanadate and

molybdate, the orange-yellow hue formed by these substances does not disappear readily, and a positive error results. If the molybdate is added to the acidified phosphate before the vanadate, yellow molybdiphosphoric acid is formed. If too much ammonium ion is present, a colloidal dispersion of ammonium molybdiphosphate may form. This precipitate does not disappear on adding vanadate. If the precipitate does not appear, the yellow solution is readily converted to molybdivanadophosphoric acid on adding vanadate.

Temperature. Most procedures specify adding the vanadate to a hot nitric acid solution, followed by cooling to room temperature before adding the molybdate. It makes no difference in the final results whether the solution is cooled to room temperature before or after adding vanadate, or after adding molybdate. A precipitate will appear, however, if the solution is boiled 15 to 20 minutes after adding molybdate.

Phosphorus Concentration. Transmittancy curves for solutions containing 1 to 100 p.p.m. of phosphorus are shown in Figure 1. Beer's law applies up to 40 p.p.m. for measurements made at 460 mμ.

Stability of the Color. The solutions used to determine the curves in Figure 1 were stored in Pyrex bottles and the transmittancies checked at measured time intervals for several weeks. Solutions containing 5 p.p.m., or more, are stable at least 7 weeks. Below this concentration, the color increases slowly, the error amounting to 2% in the transmittancy at 460 mμ in about 2 weeks.

Diverse Ions. To observe the effect of diverse ions 10 p.p.m. of phosphorus were used, the desired amount of diverse ion being included with the phosphorus solution. The apparent concentration of phosphorus was calculated from the transmittancy at 460 mμ. An error up to 2% was considered negligible.

The error does not exceed 2% for amounts up to 1000 p.p.m. of any of the following ions: aluminum, ammonium, barium, beryllium, cadmium, calcium, iron (III), lead, lithium, magnesium, manganese, mercury (I and II), potassium, silver, sodium, strontium, tin (II), uranium, zinc, zirconium, acetate, arsenite, benzoate, bromide, carbonate, chlorate, citrate, cyanide, formate, iodate, lactate, molybdate, nitrate, nitrite, oxalate, perchlorate, periodate, pyrophosphate, salicylate, selenate, silicate, sulfate, sulfite, tartrate, and tetraborate.

Copper and nickel change the hue of the solution, and thus interfere with visual comparison; but up to 1000 p.p.m. of either of these metals does not interfere with spectrophotometric measurement at 460 mμ.

Only the ceric and tin (IV) ions precipitate under the conditions used.

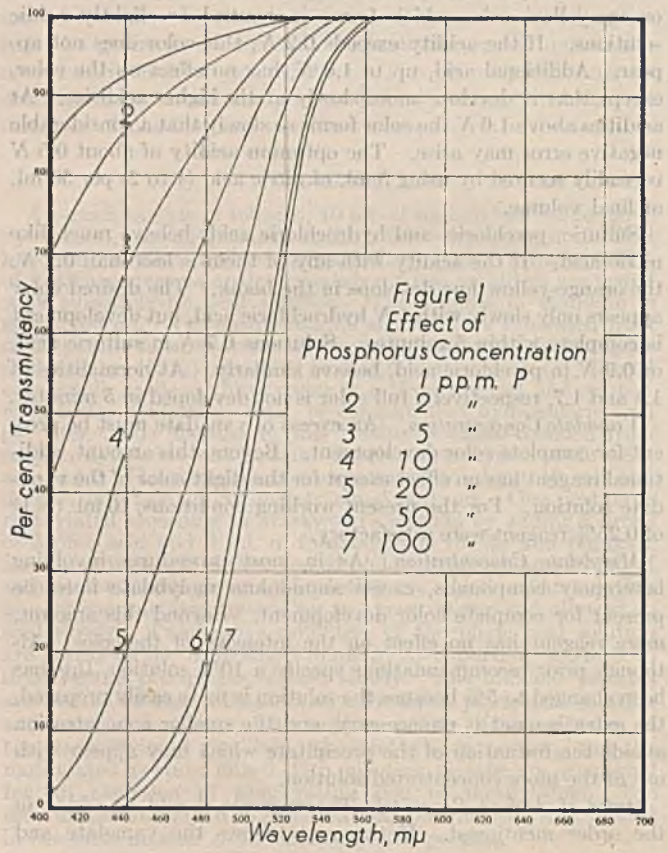
The largest single source of error is the effect of certain ions in slowing down the rate of the color reaction. Although this effect was noticed in a number of cases, generally the full color developed within 5 minutes. Bismuth, thorium, arsenate, chloride, and fluoride caused considerable negative error, however, and the full color did not develop in their presence in less than 30 minutes. The magnitude of such error may be reduced considerably by allowing the color to develop at least 30 minutes before measurement, or heating the solution in boiling water for 10 minutes after the final addition of reagent. But even then the error is not negligible if 1000 p.p.m. of the ion are present. Heating is permissible only in the absence of silicates and arsenates, since they give a positive error under these conditions.

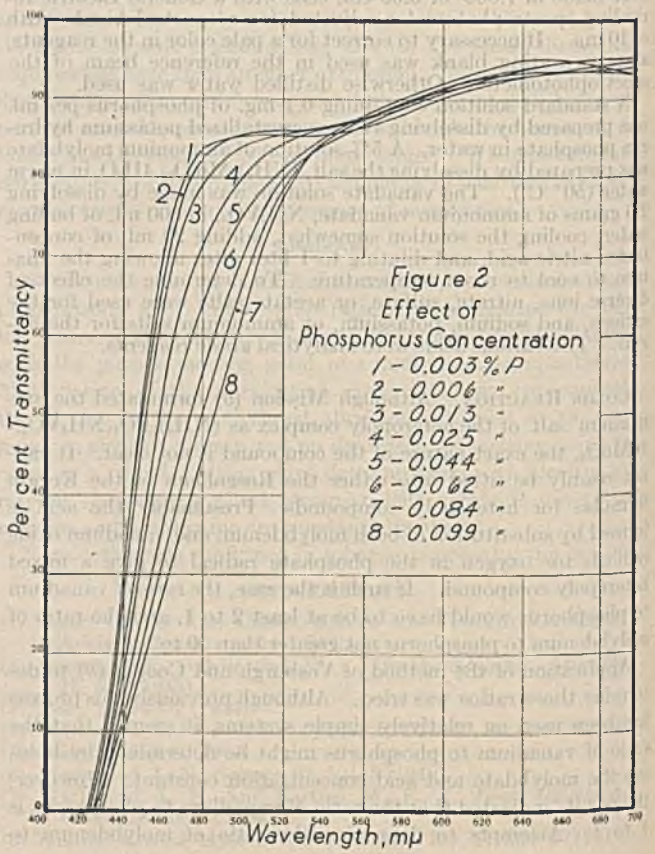
A few ions, such as iron (II), sulfide, thiosulfate, and thiocyanate, reduce the molybdivanadophosphoric acid or the excess molybdate to molybdenum blue.

The general effect of interfering diverse ions is summarized in Table I.

Discussion. Although the molybdivanadophosphoric acid method has found little application for the determination of phosphorus, the procedure is rapid, sensitive, and relatively free from interference by most common diverse ions.

Comparison of this method with the various molybdenum blue procedures summarized by Woods and Mellon (*11*) shows that it possesses several advantages. The solutions are stable at least





2 weeks, as compared with a maximum of 10 hours for any of the molybdenum blues studied. It is also relatively free from interference, as compared with them. Especially important is this characteristic for iron (III) and silicate, both of which interfere in most molybdenum blue methods. Although a molybdenum blue is more satisfactory for visual comparison than the yellow molybdivanodophosphoric acid, this technique can be used. However, photoelectric means are preferable. In general, the ranges for molybdenum blue methods are less than that for this procedure, which extends from 1 to 50 p.p.m. for 1-cm. thickness. The system conforms to Beer's law through most of this range.

Since photoelectric measurement seems preferable, a series of permanent standards was not established. Such a series could be prepared from the acid itself, but with low concentrations of phosphorus the solutions are not stable more than 2 weeks. Comparison of the transmittancy curves in Figure 1 (for 10 p.p.m.) with those for dichromate solutions (*3*) shows an approximate match with the aqueous dichromate solutions at pH 6.1.

Table I. Effect of Diverse Ions

Ion	Added as	Amount	Error	Amount Permissible
		P.p.m.	%	*P.p.m.*
Bi^{+++}	$Bi(NO_3)_3$	1000	5	400
Cr^{+++}	$Cr_2(SO_4)_3$	10	1	10
Co^{++}	$Co(NO_3)_2$	100	2	100
Fe^{++}	$Fe(NH_4)_2(SO_4)_2$	100	0	100
Th^{++++}	$Th(NO_3)_4$	400	38	20
AsO_4^{---}	Na_2HAsO_4	1000	15	125
Cl^-	NaCl	1000	24	75
$PtCl_6^{--}$	H_2PtCl_6	20	0	20
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	4	2	4
F^-	NaF	1000	51	50
I^-	KI	100	[a]	0
MnO_4^-	$KMnO_4$	10	10	0
SCN^-	KSCN	500	0	500
$S_2O_4^{--}$	$Na_2S_2O_3$	250	0	250
WO_4^{--}	Na_2WO_4	250	2	250

[a] Oxidizes to iodine.

RECOMMENDED GENERAL PROCEDURE

Sample. Procure a representative portion of the material and subject it to the necessary preparative treatment. Weigh or measure by volume a sample containing not less than 0.005 mg. of phosphorus.

If necessary, dissolve the sample by appropriate means, taking care in the dissolution and subsequent treatment to convert the phosphorus to orthophosphate. Obviously, phosphoric acid may not be used for dissolution, nor pyrophosphate for a fusion. Interfering ions should either be removed or inhibited to bring their concentrations within the limits set in Table I. Make the resulting solution just acidic to litmus and dilute to a definite volume in a volumetric flask.

Desired Constituent. Transfer a suitable aliquot of the solution to a 100-ml. flask. If Nessler tubes are used for visual comparison, at least 0.005 mg. of phosphorus should be in the aliquot. For photometric measurement in 1-cm. cells, 0.1 to 5 mg. of phosphorus should be present. Add in order, with adequate mixing, 10 ml. of nitric acid (1 to 2), 10 ml. of 0.25% ammonium vanadate solution, and 10 ml. of 5% ammonium molybdate solution. Dilute to the mark and mix well. The color may be measured at once by any of the usual means. Filter photometric measurement should be made with a blue filter having its maximum transmission near 470 mμ, and spectrophotometric measurement is made best between 460 and 480 mμ.

DETERMINATION OF PHOSPHORUS IN STEEL

Several colorimetric methods have been described for determining phosphorus in steel in a fraction of the time required for the conventional gravimetric or titrimetric procedure. A 0.5-gram sample may be handled colorimetrically with an accuracy equal to that of the usual titrimetric procedure, and with a saving of time of at least two thirds. For this purpose Mission (*6*) first used the molybdivanadophosphoric acid method. It was investigated subsequently by others (*1*, *7*, *8*). Since the procedure has not been generally applied in the routine analysis of steel, the work reported here was done to check the earlier recommendations with modern photoelectric equipment.

EXPERIMENTAL WORK

A plain carbon steel was used as the source of phosphorus in most of the work. The manufacturer's analysis gave the following percentages: C, 0.64; Mn, 0.65; Si, 0.18; S, 0.022; and P, 0.02. The last value was checked. The solutions of ammonium vanadate and molybdate were the same as used before. The potassium permanganate and the ammonium peroxydisulfate solutions were 1 and 7.5%, respectively. The 3% hydrogen peroxide was the common U.S.P. solution.

Preliminary Experiments. In most methods for the colorimetric determination of minor constituents in iron and steel, some reaction is used to convert the colored ferric ion to a colorless complex. Several common reactions are inapplicable with the molybdivanadophosphoric acid method. Phosphoric acid obviously cannot be used to form the ferric phosphate complex. Fluoride ion interferes with the color development, thus eliminating the fluoride complex. Reduction of ferric to ferrous ion is objectionable because ferrous iron partially reduces the colored complex. Although dissolution of the sample in perchloric acid gives a measurable system (*10*), the procedure recommended here proved to be more rapid and more easily controlled.

This situation necessitates developing the molybdivanadophosphoric acid color in the presence of the ferric iron color. The latter, or background color, must be reproducible to ensure reliable results in the final evaluation of the total color. Preliminary experiments with the procedure of Murray and Ashley (*7*) revealed difficulties which were traced to this background color. The deviation was small and probably would be unnoticed in various visual methods. Since it was definitely more than the 2% error considered negligible for work with the recording spectrophotometer, means were sought to reduce the uncertainty.

Subsequent work showed that a reproducible background color could be obtained with the procedure of Murray and Ashley only by careful control of the experimental conditions. The use of ammonium peroxydisulfate as oxidant eased the necessity for careful control. Solutions prepared with this reagent were more reproducible than those using the permanganate previously recommended, and they did not require such close duplication of experimental conditions. Excess oxidant is readily removed by heating, thus eliminating an operation from the procedure and saving time.

Effect of Variables on Color. The following experimental procedure was used in studying the effect of variables on the peroxydisulfate method:

A 0.5-gram sample of steel in a 150-ml. conical flask was treated with 20 ml. of nitric acid (1 to 2). After violent action ceased, the solution was heated to boiling on a hot plate and allowed to boil from 2 to 5 minutes. Five milliliters of the peroxydisulfate solution were added, and the solution was boiled from 3 to 5 minutes to destroy excess oxidant. Ten milliliters of vanadate solution were then added, and the solution was cooled to room temperature. Following addition of 20 ml. of molybdate solution, the system was mixed and transferred to a 100-ml. volumetric flask. After dilution to volume with water and mixing, the transmittancy curve was determined in 5.00-cm. cells.

Acid Concentration. The concentration of acid in the final solution is indefinite because of the amount used up in the dissolution process, and that boiled out during subsequent heating. The intense color produced with the use of small amounts of acid is probably attributable to a vanadomolybdate complex which forms at low acidity. The color with high acid concentration is due almost entirely to the iron color. The optimum amount of acid is 20 ml. of nitric acid (1 to 2). This volume provides reproducible background color, but more reduces the intensity of the final color. Measurement of the acid to ±1 ml. from a 250-ml. buret is recommended.

Peroxydisulfate Concentration. Enough ammonium peroxydisulfate must be added to oxidize organic material, but a large excess should be avoided. Although 5 ml. of a 7.5% solution were chosen as the optimum amount, twice as much is permissible. As the solution decomposes on standing, it should be prepared daily.

Vanadate and Molybdate Concentration. Variations in the vanadate and molybdate concentrations have some effect on the final color. Twenty milliliters of 5% ammonium molybdate and 10 ml. of 0.25% ammonium vanadate gave best results. These volumes should be controlled within 1 ml.

Time of Heating. The steel solution should be boiled at least 2 minutes after dissolution of the sample, but boiling as long as 5 minutes has no deleterious effect on the color. The length of time of boiling after adding peroxydisulfate is apparently not critical, since variation from 3 to 10 minutes made no difference.

Order of Adding Reagents. Three successions of adding reagents were tried: both the vanadate and the molybdate to the hot steel solution; the vanadate to the hot solution, followed by cooling before adding the molybdate; and both the molybdate and vanadate to the cooled steel solution. The same final color was obtained. However, the second order was finally adopted, chiefly because of previous recommendation.

Stability of Color. The full color intensity develops immediately on adding the molybdate, and the color is stable for at least an hour. Since the method is being recommended for rapid photometric measurement, this factor was not studied further.

RECOMMENDED PROCEDURE FOR STEELS

Based on this experimental evidence, the following procedure is recommended for the determination of phosphorus in plain carbon and certain low alloy steels:

SAMPLE. Weigh a 0.5-gram representative sample into a 150-ml. conical flask, add 20 ml. of nitric acid (1 to 2), and, after violent action ceases, boil 2 minutes to remove nitrous oxide fumes. Add 5 ml. of a freshly prepared 7.5% solution of ammonium peroxydisulfate, and boil 3 to 5 minutes to destroy the excess.

DESIRED CONSTITUENT. Add 10 ml. of 0.25% ammonium vanadate solution to the hot solution and then cool to room temperature. After adding 20 ml. of 5% ammonium molybdate solution, mix thoroughly and transfer the solution to a 100-ml. volumetric flask. Dilute to the mark, mix, and measure the color intensity by any suitable means. A blue filter with maximum transmission near 470 mμ is suitable for a filter photometer. With a spectrophotometer the best wave length is the range 460 to 480 mμ.

Table II. Data Used for Calibration Curve

Steel No.	Phosphorus	T_{460}	Log T_{460} Observed	Log T_{460} Calcd.[a]
	%	%		
55	0.003	68.8	1.838	1.843
11d	0.006	68.0	1.833	1.830
12d	0.013	62.9	1.799	1.798
16b	0.025	55.5	1.744	1.744
20c	0.044	45.9	1.662	1.658
21c	0.062	36.8	1.566	1.576
22b	0.084	31.2	1.494	1.476
8d	0.099	25.0	1.398	1.409

[a] Calculated from the equation

$$\% \text{ P} = \frac{1.857 - \log T}{4.52}$$

ANALYSIS OF STANDARD SAMPLES

The final test of the procedure was the analysis of some 20 plain and low-alloy steels from the National Bureau of Standards. Eight plain carbon steels of different phosphorus contents were used to establish a calibration curve. The remainder were analyzed on the basis of this curve. Three samples of each steel were prepared according to the recommended procedure. The average of the transmittancy readings at 460 mμ was used as the most probable value.

The data secured for the eight steels used for the calibration curve are summarized in Table II. The transmittancy curves for the solutions are shown in Figure 2. If log T_{460} is plotted against percentage of phosphorus, a straight line is obtained, indicating conformity to Beer's law. The equation of the best straight line was calculated by the method of least squares, the data obtained being included in Table II.

After the calibration curve had been established, 14 plain carbon and low-alloy steels were analyzed (Table III). In each case the average value found is the result of at least 3 determinations. The average deviation from the mean was usually less than 2 to 3% of the phosphorus present.

Table III. Analysis of Standard Steels

Sample Type and No.		Phosphorus Found	Deviation from Standard	Allowable Deviation (δ)
		%	%	%
Bessemer	9c	0.091	−0.005	±0.004
Bessemer	10d	0.087	0.001	0.004
Bessemer	23a	0.102	0.000	0.004
B.O.H.	13c	0.011	−0.002	0.002
B.O.H.	15a	0.006	−0.001	0.002
A.O.H.	21a	0.062	−0.001	0.003
A.O.H.	34	0.095	0.000	0.004
A.O.H.	35	0.035	+0.002	0.003
Electric	51	0.016	+0.005	0.002
Acid electric	65a	0.036	0.000	0.003
Mn rail	100	0.022	−0.001	0.002
Ni	33b	0.036	−0.001	0.003
Ni-Cr	32b	0.024	+0.008	0.002
Cr-Mo	72	0.033	+0.017	0.002

Examination of the results shows that in four cases the experimental results are outside the limits set for gravimetric or titrimetric methods. The reason for the deviation in steel 9c is unknown, three excellent checks being obtained. With steel 51, however (because of high carbon ?), 6 samples diverged widely. Other samples in which deviation occurred contained moderate amounts of chromium, which would be expected to interfere.

The accuracy of the calibration curve was confirmed by calculating the best straight line for all the steels except Nos. 32b, 33b, 51, and 72. The formulas for the best straight lines are

$$\% \text{ P} = \frac{1.857 - \log T_{460}}{4.529} \text{ (8 samples)}$$

$$= \frac{1.857 - \log T_{460}}{4.500} \text{ (18 samples)}$$

DISCUSSION. The use of ammonium peroxydisulfate has several advantages over potassium permanganate in this method. The background color is more nearly reproducible. Since exact control of experimental conditions is not necessary, the over-all procedure is simplified. There is a saving of approximately 10 minutes in time.

The calibration curve may be prepared by using a series of steels of known phosphorus content, or by adding known amounts of phosphorus to solutions of a single analyzed steel. The former alternative was used in this work, and the latter by Murray and Ashley (7). Their method seems preferable if one type of steel is to be analyzed, since the background color is then essentially constant. As Beer's law applies to the system, one may extrapolate to concentrations of phosphorus below that of the standard. If several types of steel, having compositions not too different but giving different background colors, are to be analyzed, an average calibration curve should be prepared from a series of known steels of the types to be analyzed. In any questionable case, the second alternative should be used. Instead of taking the results from such a calibration curve, they may be calculated from the straight-line equation already mentioned.

Murray and Ashley reported serious interference with high (4%) silicon, but in the usual range of this element no interference was found in this work. More than 0.4% chromium gives a positive error. In general, the accuracy equals that of the common titrimetric method, which is less rapid and requires more skill. The molybdenum blue method of Hague and Bright (*2*) is also less rapid.

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ABSTRACTED from a portion of the thesis presented by R. E. Kitson to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of doctor of philosophy.

Determination of Potassium in Fertilizer Mixtures

Removal of Ammonia and Organic Matter without Ignition

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THE ignition step in the A.O.A.C. (*1*) method for the determination of potassium in mixed fertilizers requires careful attention and may sometimes result in potassium losses or insoluble residues. This paper presents a procedure which has been found to reduce errors and shorten the time of analysis of many fertilizers by eliminating high-temperature ignition with sulfuric acid.

Kraybill and Thornton (*4*) have called attention to losses during ignition which may be caused by spattering or volatilization. St. John and Midgley (*6*) found that controlled temperatures tend to avoid volatilization of potassium due to localized overheating. They also noted insoluble residues from ashing plant materials with sulfuric acid, but usually none when using their acid digestion method.

In his 1940 Report on Potash, Ford (*3*) showed that errors from "water-insoluble residues that are often encountered" should be corrected either by filtering the potassium solution before adding platinic chloride, or by dissolving the weighed potassium platinic chloride with hot water and reweighing the dried crucible. In either case additional work is involved which increases the time of analysis.

In 1934 this laboratory experienced difficulty in obtaining accurate potash results by the official method of that time when analyzing fertilizers containing little or no superphosphate and large amounts of monoammonium phosphate. Since the ignition step was found to be the main source of error in the analysis of mixtures of this type (*2*), a procedure was adopted which eliminated ignition by using a low-temperature method to remove interfering ammonium salts and organic matter. Thus it was impossible to form insoluble metasilicates or phosphates.

Changes in the A.O.A.C. method were made in 1935, so that with proper manipulation and corrections for insoluble residue it was possible to determine potassium in all types of fertilizer with a greater degree of accuracy. Although these changes included better ignition technique, the low-temperature procedure as used in this laboratory appeared to have certain advantages. Several improvements in the method have been made recently and analytical results compared with those obtained by ignition with sulfuric acid, using the same solution for both determinations.

METHOD

REAGENTS (other than used in A.O.A.C. method). Concentrated sodium hydroxide solution, 30 grams of A.C.S. reagent sodium hydroxide per 100 ml. of solution. Sodium chlorate solution, 10 grams of A.C.S. reagent sodium chlorate per 100 ml. of solution. 30% sulfuric acid, 20 ml. of sulfuric acid (A.C.S. reagent, 1.84 sp. gr.) in 80 ml. of water.

METHOD 1 (for inorganic fertilizers). Place 5 grams of sample in a 250-ml. volumetric flask and add about 100 ml. of water and 50 ml. of saturated ammonium oxalate solution. Boil 30 minutes, cool, dilute to 250 ml., mix well, and filter or allow coarser particles to settle. Pipet a 100-ml. aliquot into a 500-ml. Kjeldahl flask containing a few glass beads. (If moisture content of the fertilizer permits grinding fine enough to prevent separation of particles, weigh a 2-gram sample directly into the Kjeldahl flask. Add about 75 ml. of water and 20 ml. of saturated ammonium oxalate solution. Boil 10 minutes.)

Add 3 or 4 drops of 1% phenolphthalein solution and 5 ml. of concentrated sodium hydroxide solution. Boil vigorously until damp red litmus paper placed over mouth of flask shows no trace of blue color when left there several minutes. The time required to expel ammonia is usually less than 15 minutes. If red color of phenolphthalein fades during boiling, add sufficient sodium hydroxide to restore.

Cool and transfer with thorough washing to a 200-ml. volumetric flask. Dilute to mark, mix well, and pass through a close-textured dry filter. Determine K_2O on a 25- or 50-ml. aliquot by the platinic chloride method. Treat precipitate in evaporating dish with about 15 ml. of 85% alcohol and 1 ml. of concentrated hydrochloric acid. Use rubber policeman to break up residue thoroughly before transferring to Gooch crucible. Wash as usual with alcohol and ammonium chloride solution, but use ten 10-ml. portions of ammonium chloride solution if a 50-ml. aliquot was taken. Adjust suction during the ammonium chloride washing so that the jet from wash bottle or 10-ml. pipet (enlarged tip opening) will about half fill the crucible and agitate the precipitate with each washing.

METHOD 2 (for fertilizers containing urea, cyanamide, or other interfering organic materials). Prepare a 5-gram to 250-ml. fertilizer solution as in Method 1, but after boiling 30 minutes add 2 ml. of concentrated ammonium hydroxide or sufficient to make alkaline. Cool, dilute to volume, mix well, and pass through a close-textured dry filter. Add 5 ml. of concentrated nitric acid to a 100-ml. aliquot in a 500-ml. Kjeldahl flask and boil vigorously to a volume of about 10 ml. Add 5 ml. of sodium chlorate solution and 5 ml. of 30% sulfuric acid. (If sample is high in organic matter or urea, use 10 ml. of 30% sulfuric acid.)

Boil to dense sulfur trioxide fumes using low heat, then use high heat for 10 to 15 minutes to destroy organic matter. Cool 3 or 4 minutes and wash down neck of flask with about 100 ml. of water. Heat to obtain complete solution of residue. Add phenolphthalein and neutralize with concentrated sodium hydroxide solution. Add 2 ml. excess and boil to negative test for ammonia. Proceed as in Method 1.

DISCUSSION

The use of a 5-gram sample in this method rather than the official 2.5 grams is based upon the study of Kraybill and Thornton (*5*), who found that errors may result due to difficulty in weighing uniform 2.5-gram samples. The larger sample is taken in order to minimize such errors, usually caused by separation

Table I. Comparative K_2O Analyses and Composition of Laboratory Mixtures

Mixture[a]	Calculated K_2O %		H_2SO_4 Ignition K_2O %	New Method K_2O %	Composition of Mixture %
L-1	22.2		22.14	22.19	20 KCl[b]
			22.32	22.23	20 K_2SO_4[c]
					10 Superphosphate
					25 $(NH_4)_2SO_4$
					20 Ammo-Phos A
		Av.	22.23	22.21	5 Bone meal
L-2	22.2		21.93[d]	22.17	20 KCl[b]
			22.24	22.10	20 K_2SO_4[c]
					10 Superphosphate
					10 Ammo-Phos A
					10 $CaCN_2$
					10 Uramon
					10 Fish meal
		Av.	22.09	22.14	10 Bone meal
L-3	30.4		30.28	30.35	50 KCl[e]
			30.44	30.30	10 Superphosphate
					20 $(NH_4)_2SO_4$
					10 Ammo-Phos A
		Av.	30.36	30.33	10 Tankage
L-4	30.4		30.37	30.42	50 KCl[e]
			30.43	30.36	10 Superphosphate
					20 $(NH_4)_2SO_4$
					10 Ammo-Phos A
		Av.	30.40	30.39	10 Uramon
L-5	30.4		30.18	30.41	50 KCl[e]
			30.40	30.29	20 Superphosphate
					20 $(NH_4)_2SO_4$
		Av.	30.29	30.35	10 Ammo-Phos A
L-6	10.8		10.75	10.76	20 K_2SO_4 c.p.[f]
					40 $NaNO_3$
					40 $(NH_4)_2SO_4$

[a] Materials weighed on analytical balance. Duplicate analyses made on a second series of weighings.
[b] Trona muriate. Analysis by this laboratory, 61.29% K_2O. Manufacturer's analysis, 61.26% K_2O.
[c] Foreign potassium sulfate. Analysis by this laboratory, 49.70% K_2O. Manufacturer's guarantee, 49.5% K_2O.
[d] Low result probably caused by spattering at start of ignition due to large amount of organic matter.
[e] Trona muriate. Analysis by this laboratory, 60.80% K_2O. Manufacturer's analysis, 60.81% K_2O.
[f] Analytical reagent, dried before using. Theoretical K_2O, 54.05%.

of coarse 1-mm. particles from the fines. Many fertilizers of low moisture content, however, can be finely ground and have only traces of organic matter from phosphates and by-product ammonium sulfate. Two grams of such material may be treated directly for ammonia removal by the shortened procedure. The total time from weighing a 2-gram sample to taking an aliquot for evaporation with platinic chloride is about 40 minutes.

The time of boiling a 5-gram sample with ammonium oxalate has been made to agree with the A.O.A.C. period of 30 minutes, but it has been the experience of this laboratory that 10 to 15 minutes is sufficient for most types of fertilizer, especially when the composition of the sample is known or a finely ground 2-gram sample is used.

Although Method 1 does not require addition of ammonium hydroxide after boiling with ammonium oxalate, this may be done if the solution is to be used for a check analysis by sulfuric acid ignition. This does not appreciably lengthen the time of expelling ammonia from a 100-ml. aliquot.

Phenolphthalein indicator is not destroyed or volatilized during the boiling period. Fading of color indicates a drop in pH and will occur only in exceptional cases where 5 ml. (1.5 grams) of sodium hydroxide are not sufficient to react with all ammonium salts present. If it is desired to check the litmus test for complete removal of ammonia, phenolphthalein may be omitted and a portion of the final filtered solution treated with Nessler reagent.

The final solution of a fertilizer analyzed by Method 1 will contain a small amount of sodium oxalate. In case the sample contains no soluble calcium the amount of sodium oxalate in a 50-ml. aliquot taken for evaporation with platinic chloride will be approximately 0.24 gram. This may be decomposed before precipitation of potassium platinic chloride by the hypochlorite reaction, or preferably allowed to remain with the precipitate, since this amount of sodium oxalate is easily removed by acid alcohol and ammonium chloride washing. Decomposition by sodium hypochlorite is accomplished by the addition of 3 to 4 ml. of pure 5% available chlorine solution to a 50-ml. aliquot in a porcelain dish. The solution is then made distinctly acid with concentrated hydrochloric (2 ml.) and evaporated for 10 minutes, after which the necessary amount of platinic chloride is added.

The final solutions of organic fertilizers will not contain oxalate since it is destroyed by the sulfuric acid treatment of Method 2. In this procedure sodium chlorate is used in addition to sulfuric acid in order to destroy other forms of organic matter, especially when present in large amounts. No attempt was made to use perchloric acid because of the possible hazard of explosion.

Table I gives the comparative analyses and composition of six fertilizer mixtures made in the laboratory, each containing known amounts of K_2O. Interfering materials such as calcium cyanamide, urea, tankage, and fish meal were used in amounts greater than those ordinarily found in commercial fertilizers. One inorganic mixture (L-6) which contained no calcium was included, so that the maximum amount of sodium oxalate would be present.

In these particular mixes, with the exception of L-1, ignition with sulfuric acid according to the A.O.A.C. technique produced only traces of insoluble residue. The asbestos-padded Gooch crucibles were therefore not washed out with hot water and reweighed. Table II gives comparative analyses on various types of local commercial fertilizers.

Table II. Comparative K_2O Analyses of Commercial Fertilizers

Formula	H_2SO_4 Ignition K_2O %	New Method K_2O %	Variation K_2O %
7-20.5-17	18.07	18.02	−0.05
7-20.5-17[a]	16.48	16.49	+0.01
7-20.5-17[b]	17.86	17.80	−0.06
6-20-12	13.32	13.31	−0.01
8-12.5-6	5.72	5.77	+0.05
7-3.5-23	23.01	23.15	+0.14
6-15-10	9.98	9.91	−0.07
7-10-10	10.17	10.23	+0.06
7-11-10	10.48	10.50	+0.02
8-9-11	10.68	10.59	−0.09
7-12-7	7.83	7.79	−0.04
11-20-22	26.63	26.61	−0.02

[a] Containing 252 pounds of bone meal per ton.
[b] Containing 50 pounds of Uramon per ton.

SUMMARY

A method presented for the determination of potassium in mixed fertilizers involves the usual ammonium oxalate solution and chloroplatinic acid precipitation, but employs a rapid volatilization procedure for removal of ammonia, and an oxidizing method for destroying interfering organic compounds. This means of preparing a solution from which potassium may be precipitated makes it unnecessary to ignite at a high temperature with sulfuric acid, which may result in the formation of insoluble residue or the loss of potassium by spattering or volatilization.

A large number of analyses by this method have been made on inorganic and organic fertilizers containing salts of known potassium content, and the results are in close agreement with the calculated values of these mixtures.

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Relationship between Unsaturation and the Ultraviolet Absorption Spectra of Various Fats and Fatty Acids

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The spectral absorption of several unsaturated fatty acids and natural fats have been measured from 2500 to 2100 Ångstrom units. Data are presented that show a definite relationship between the degree of unsaturation and extinction coefficients at 2100 Ångstrom units. From the composition of natural fats it is possible to predict the extent of absorption at this wave length.

IT IS well known that absorption by the carbon to carbon double bond, one of the most important chromophores (*3, 6*), is modified by factors such as the cis- and trans-configuration, weighting by substituent groups, the number of double bonds in the carbon chain, and their positions relative to each other (*3*). The natural fatty acids, their esters, and isomers constitute a most important group of aliphatic compounds differing chiefly in the number and position of double bonds. In a recent review (*2*) it was pointed out that except for the saturated fatty acids (*13*) the absorption curves of the well-known members of this series of compounds and the natural oils have usually not been extended below 2200 or 2300 Å. (*1, 4, 5, 7, 9, 14, 19*). The measurements that have been made in the longer wave lengths show such great irregularities that it must be assumed that impurities with strong absorption bands are affecting the results.

The effect of increasing numbers of unconjugated double bonds in simple hydrocarbons is so marked at 2100 Å. (*3, 6*) that it was decided to study the absorption spectra of the highly purified fatty acids. Although it was not possible to extend the curves of the unsaturated acids to their maxima (below 2000 Å.) because of the limit of the spectrograph (2100 Å.), there nevertheless was found a large and consistent effect of increasing unsaturation which seemed of practical importance, since this wave length is within the range of many spectrographs now in use.

EXPERIMENTAL

The absorption measurements from 2100 to 2250 Å. were were made with a Gaertner Littrow spectrograph. From 2300 to 2500 Å. absorption was measured with a photoelectric spectrophotometer similar to that described by Hogness *et al.* (*8*). The solvent employed was purified ethyl alcohol (commercial 95% alcohol freshly distilled from potassium hydroxide) for all samples except stearic acid which was dissolved in ethyl ether (freshly opened anesthesia grade). The absorption values of the pure compounds are plotted as the logarithm of the molecular extinction coefficients, ϵ, while the values for the oils are expressed as $E^{1\%}_{1\,\text{cm.}}$, where 1% means 1 gram in 100 cc. of solution. The values were calculated from Lambert's and Beer's law,

$$\log \frac{I_0}{I} = \epsilon c l$$

Measurements were made on samples of the highest purity obtainable. (The authors are indebted to J. P. Kass and J. Nichols for the preparation of these materials.) The stearic acid melted at 69.6° in a capillary tube and had no measurable iodine number. Oleic acid was prepared by repeated recrystallization at low temperature of the fatty acids of olive oil until a sample with iodine number (Wijs) of 88 was obtained. The chief impurity probably was palmitic acid. The methyl esters of linoleic, linolenic, and arachidonic acids were made from the recrystallized polybromides by debromination in methyl alcohol. The iodine number of each preparation was within 2 units of the theoretical value.

[1] Deceased.

The curves in Figure 1 show the marked effect of unsaturation on spectral absorption below 2250 Å. At 2100 Å. the long-chain fatty acids have the following molecular extinction coefficients: stearic 60; oleic 180; methyl linolate 2500; methyl linolenate 10,000; and methyl arachidonate 14,500. In other words, arachidonic acid with an iodine number approximately 4 times that of oleic acid has a spectral absorption at 2100 Å. which is roughly 80 times as great.

The suggestion of an absorption band at 2350 Å. may be due to trace impurities. This is the region of maximum absorption by conjugated dienes and it is known that in the saponification, bromination, debromination, and distillation of highly unsaturated fatty acids some conjugation may take place (*16, 17, 18*). However, since the conjugated dienes have molecular extinction coefficients of 20,000 to 30,000 in this region (*2*), there could not be more than a fraction of 1% present in any of these preparations. This would not measurably affect the absorption values of the highly unsaturated acids at 2100 Å. although it is sufficient to throw the curves out of line at 2300 Å.

The large and regular effect of unsaturation on light absorption at 2100 Å. is contrasted with the smaller and irregular effects at the longer wave lengths in Figure 2. It is clear that if absorption at 2100 Å. can be measured with sufficient accuracy the values can be used as constants in simultaneous equations for calculating the fatty acid composition of oils. This direct measurement may well be used instead of the one described by Kass *et al.* (*12*) and later extended by Mitchell *et al.* (*15*), which depends upon the measurement of the conjugated linoleic and linolenic acids after saponification at a high temperature. The chief disadvantage of the present method comes from the requirement that the measurements be made at a wave length shorter than that reached by many instruments.

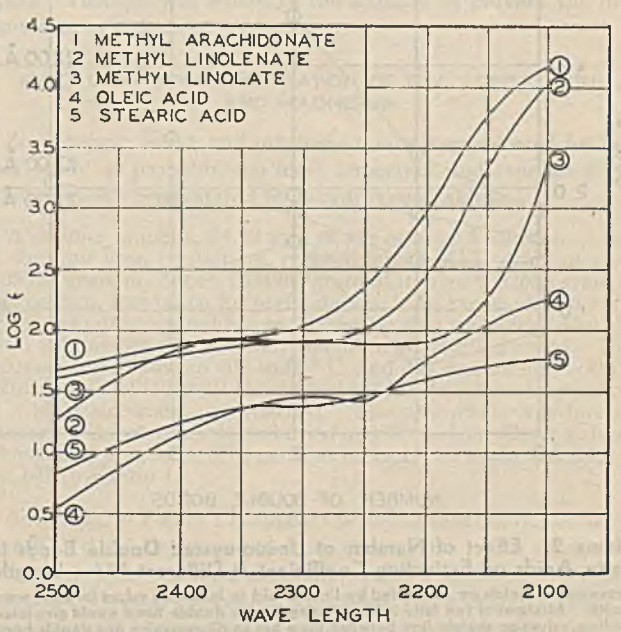

Figure 1. Absorption Spectra of Five Fatty Acids with Different Numbers of Unconjugated Double Bonds

Table I. Comparison of Calculated and Experimentally Determined Extinction Coefficients for Natural Fats

Fat	Iodine Value (Wijs)	Thiocyanogen Value	Saturated Acids	Oleic Acid	Linoleic Acid	Extinction Coefficients, $E^{1\%}_{1\,cm.}$ at 2100 Å. Calculated	Measured
Coconut	8.6	7.3	87.4	6.6	1.5	3.8	5.9
Olive	83.2	74.8	11.8	74.0	9.7	15.2	18.2
Corn (Mazola)	134.2	82.8	6.5	29.6	59.4	61.6	60.0

In Figure 3 the curves of four plant fats of widely different composition are compared with those of the fatty acids. The extinction coefficients, $E^{1\%}_{1\,cm.}$, at 2100 Å. are in the range that would be expected from the composition of the fats. Iodine numbers (Wijs) and thiocyanogen numbers were determined for coconut oil, olive oil, and corn oil. (The authors are in debted to H. G. Loeb for these determinations.) From these analytical constants the composition of the three fats was calculated (Table I). The corrected value for the thiocyanogen number of linoleic acid as given by Kass *et al.* (*11*) was substituted in Equation 3 of the following simultaneous equations described by Jamieson (*10*):

$$x + y + s = 1 \quad (1)$$

$$86.01\,x + 173.20\,y + o = I.N. \quad (2)$$

$$86.01\,x + 90.59\,y + o = T.N. \quad (3)$$

where x is the amount of oleic acid glyceride; y, the linoleic acid glyceride; and s, the saturated acid glyceride present in the fat. After determining the composition of the fat, and converting to the free acids (95.5% of the glycerides) the values were substituted in the equation:

$$7.1\,x + 100\,y + 2.1\,s = E^{1\%}_{1\,cm.}$$

The $E^{1\%}_{1\,cm.}$ at 2100 Å. for oleic acid is 7.1; for linoleic acid, 100; and for stearic acid, 2.1. The $E^{1\%}_{1\,cm.}$ calculated in this manner was then compared with the value determined experimentally. The results (Table I) are seen to be of the right order of magnitude. Both coconut oil and olive oil have very low spectral absorption at 2100 Å. and are thus subject to considerable error introduced by traces of highly absorbing materials. A small error in the calculation of the linoleic acid content would also have a large effect. For example, if the coconut oil really contained 2.5% linoleic acid instead of the calculated 1.5%, the $E^{1\%}_{1\,cm.}$ would be raised to 5.6. However, corn oil absorption is of such magnitude that the effect of contamination is minimized and, consequently, it is possible to show a close agreement between the calculated and experimental values for this fat.

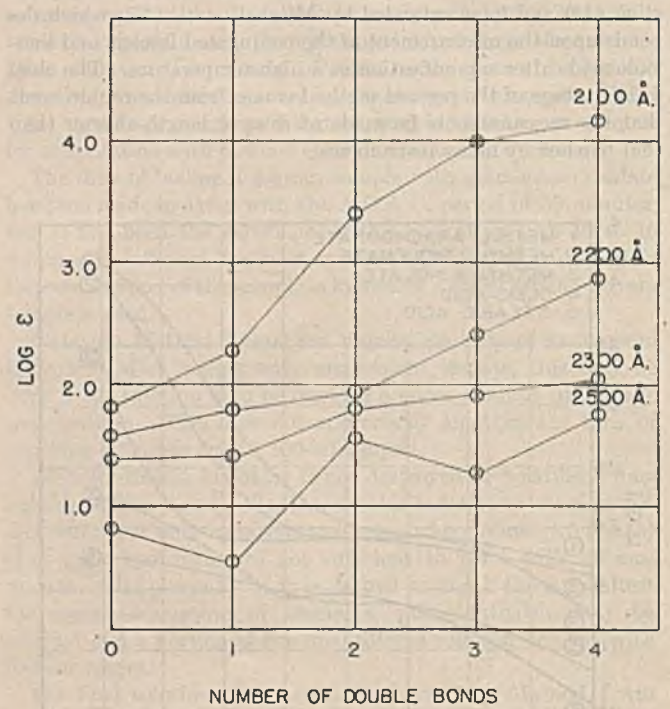

Figure 2. Effect of Number of Unconjugated Double Bonds in Fatty Acids on Extinction Coefficient at Different Wave Lengths

Experimental points are connected by lines to aid in following values for same wave length. Mixtures of two fatty acids differing by one double bond would give intermediate values on straight line between them but an oil averaging one double bond by having an equal amount of saturated acid and linoleic acid would not have absorption of oleic acid glyceride (1 double bond)

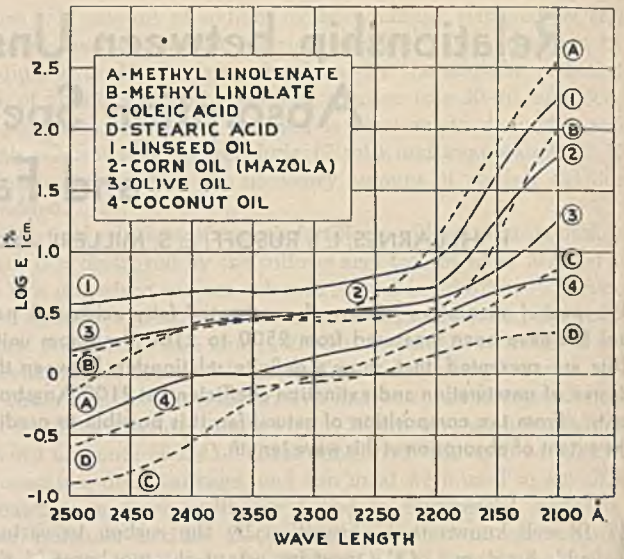

Figure 3. Absorption Spectra of Four Vegetable Oils Compared with Their Constituent Fatty Acids

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8-Hydroxyquinaldine as an Analytical Reagent

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8-Hydroxyquinaldine is a more selective reagent than 8-hydroxyquinoline because it does not precipitate aluminum. Separations of zinc from magnesium, from aluminum, and from magnesium and aluminum are given. The precipitates of the zinc and the magnesium complex salts may be either weighed or determined volumetrically by bromination. Aluminum may be determined in the filtrate of the zinc determination by adding 8-hydroxyquinoline. The effect of the pH upon the completeness of precipitation of the 8-hydroxyquinaldine complexes of cupric, zinc, ferric, and magnesium ions has been studied. Complete directions for an improved method of preparing 8-hydroxyquinaldine are given.

MANY derivatives of 8-hydroxyquinoline, "oxine", have been prepared and their analytical uses have been investigated (*7*). Most of these derivatives have been 5-, 7- or 5,7 substitution products. Although several 2-substituted derivatives are known, apparently only one, 2-phenyl-8-hydroxyquinoline-4-carboxylic acid, has been tested for analytical purposes (*1*). Since the 2-methyl-8-hydroxyquinoline or 8-hydroxyquinaldine has been known for some time (*2*) and is one of the simplest 2-substituted derivatives, the authors choose to investigate it first.

As compared to 8-hydroxyquinoline, 8-hydroxyquinaldine exhibits some important differences in behavior. Probably because of its increased size, it is a more selective reagent. If size of the molecule is a determining factor, the larger molecule might be expected not to react with the smaller ions because of the difficulty in grouping three large molecules around the small ion. If the complex is formed, it might be less stable. This is supported by the fact that 8-hydroxyquinaldine does not react with aluminum ions, one of the smallest trivalent ions (*3*) with which 8-hydroxyquinoline reacts, and the 8-hydroxyquinaldine complex with ferric ion is precipitated completely only in a much less acid solution than that required by 8-hydroxyquinoline.

REAGENTS

2-METHYL-8-HYDROXYQUINOLINE. The original method of Doebner and v. Miller (*2*) was modified as suggested by Kochendoerfer (*5*). Fifty-five grams (0.50 mole) of *o*-aminophenol and 25 grams (0.18 mole) of *o*-nitrophenol were dissolved in 100 grams of 12 *N* hydrochloric acid in a three-necked flask fitted with reflux condenser, mechanical stirrer, and dropping funnel. Forty grams (0.57 mole) of crotonaldehyde were added with stirring over a period of about 45 minutes. The mixture was heated on the steam bath for 6 hours with continual stirring and was then allowed to stand overnight. The excess *o*-nitrophenol was removed by steam-distillation from the acid solution. Fourteen grams of *o*-nitrophenol were recovered.

The residue was nearly neutralized with 6 *N* sodium hydroxide solution and then saturated with sodium carbonate and steam-distilled. The yield of crude 8-hydroxyquinaldine was 24 to 32 grams or 30 to 40%.

Five grams of the crude material were distilled under reduced pressure (water pump) in a sublimation tube and 4.9 grams of light red material were obtained. The 8-hydroxyquinaldine (4.9 grams) was crystallized from a mixture of 20 ml. of 95% ethyl alcohol plus 10 ml. of water and 4.1 grams of a slightly yellow product were obtained, m.p. = 69° C. This product is pure enough for analytical use but was recrystallized once again for the authors' experiments: m.p. = 72° C. (literature, *2*, 74° C.) Some crude material can be recovered from the mother liquors.

The reagent solution is prepared by dissolving 5 grams of 8-hydroxyquinaldine in 12 grams of glacial acetic acid and diluting to 100 ml. with water. An alcoholic solution is prepared by dissolving 5 grams in 100 ml. of 95% ethyl alcohol. Alcoholic solutions of the reagent turn dark in 1 to 2 days and should be freshly prepared. An acetic acid solution is stable for a week or longer.

STANDARD SOLUTIONS. Standard solutions of iron, copper, and zinc were prepared by dissolving weighed samples of the pure metals in acid and diluting to volume in a volumetric flask. Copper was dissolved in nitric acid and evaporated with sulfuric acid to obtain the sulfate. Iron was dissolved in sulfuric acid and oxidized to the ferric state with nitric acid, the excess of which was later evaporated off. Zinc was dissolved in hydrochloric acid.

A standard solution of magnesium sulfate was prepared by dissolving a weighed amount of C.P. magnesium sulfate heptahydrate in a measured amount of water. The solution was further standardized by gravimetric precipitation of the magnesium as magnesium ammonium phosphate and ignition to the pyrophosphate and by precipitation of the 8-hydroxyquinoline salt.

A standard solution of aluminum ion was prepared by dissolving C.P. potassium alum in a measured amount of water.

STANDARD POTASSIUM BROMATE SOLUTION. A standard potassium bromate solution, approximately 0.1 *N*, was prepared by dissolving a weighed amount of C.P. potassium bromate, dried at 110° C., in water and diluting to the mark in a volumetric flask. It was further standardized against Bureau of Standards arsenious oxide, using methyl orange as indicator.

STANDARD SODIUM THIOSULFATE SOLUTION. C.P. sodium thiosulfate was dissolved in distilled water to make an approximately 0.05 *N* solution, which was standardized against the potassium bromate solution.

AMMONIUM ACETATE SOLUTION, 2 *N*. This was prepared by dissolving 154 grams of C.P. ammonium acetate in water and diluting to 1 liter.

QUALITATIVE REACTIONS OF 8-HYDROXYQUINALDINE

Qualitative tests were performed upon most of the common ions as listed below. In acetic acid–acetate buffered solutions, 8-hydroxyquinaldine forms precipitates with Bi^{+++}, Cd^{++}, Cr^{+++}, Co^{++}, Cu^{++}, Fe^{++}, Fe^{+++}, Mn^{++}, Ni^{++}, Ag^{+}, TiO^{++}, Zn^{++}, MoO_4^{--}, WO_4^{--}, and VO_3^{-}. It does not form a precipitate with Be^{++}, Al^{+++}, Ca^{++}, Sr^{++}, Ba^{++}, Pb^{++}, Mg^{++}, K^{+}, Na^{+}, or NH_4^{+}. It does not precipitate Bi^{+++} or Sn^{++++} in tartrate solutions.

In ammoniacal solutions, the ions precipitated in acetic acid–acetate solutions, with the exception of MoO_4^{--}, WO_4^{--}, and small amounts of VO_3^{-}, are precipitated and, in addition, Pb^{++}, Mg^{++}, Ca^{++}, and Sr^{++}. Aluminum ions are still not precipitated. Tartrate was added to the solution to prevent the precipitation of aluminum hydroxide.

EFFECT OF pH UPON PRECIPITATION OF ZINC, COPPER, IRON, AND MAGNESIUM

Zinc, cupric, ferric, and magnesium ions were selected for further study as probably the most important and representative ions which are precipitated by 8-hydroxyquinaldine.

A definite amount, 24.99 ml., of the standard solution of one of the four ions, containing, respectively, 0.05121 gram of zinc, 0.05028 gram of copper, 0.04965 gram of iron, or 0.02558 gram of magnesium, was taken for precipitation. An excess of 1 to 2 ml. of 5% 8-hydroxyquinaldine in 2 *N* acetic acid was added and the total volume brought to about 200 ml. with distilled water. The solution was heated to 60° to 80° C. and 2 *N* ammonium acetate solution was added until the desired pH, as determined by means of a glass electrode, was reached. The precipitate was filtered through a Gooch crucible and dried at 120° to 130° C. for at least 3 hours and weighed. The per cent precipitated is plotted against the pH in Figure 1.

According to Figure 1 it appears probable that cupric, ferric, or zinc ions could be separated from magnesium. The separation of each of these ions from aluminum is also a possibility with this reagent. The ferric complex with 8-hydroxyquinaldine requires a considerably higher pH for complete precipitation than the corresponding 8-hydroxyquinoline complex (*4*, *6*).

RECOMMENDED PROCEDURES

Zinc can be separated from aluminum and magnesium ions by precipitation in acetic acid–acetate solutions with 8-hydroxyquinaldine. The zinc can be determined gravimetrically by weighing the precipitate or volumetrically by bromination. If aluminum is present, tartrate is added to prevent precipitation of any basic aluminum salts.

Magnesium can be determined in the filtrate from the zinc determination, if no aluminum is present, by raising the pH to 9.3 or higher. When tartrates and a high concentration of ammonium salts are present (when aluminum is present) the magnesium-8-hydroxyquinaldine complex precipitates so slowly that the method is useless. Calcium ions interfere in the magnesium determination in amounts over 2 or 3 mg. and should be previously removed.

Aluminum can be determined after removal of the zinc by adding 8-hydroxyquinoline.

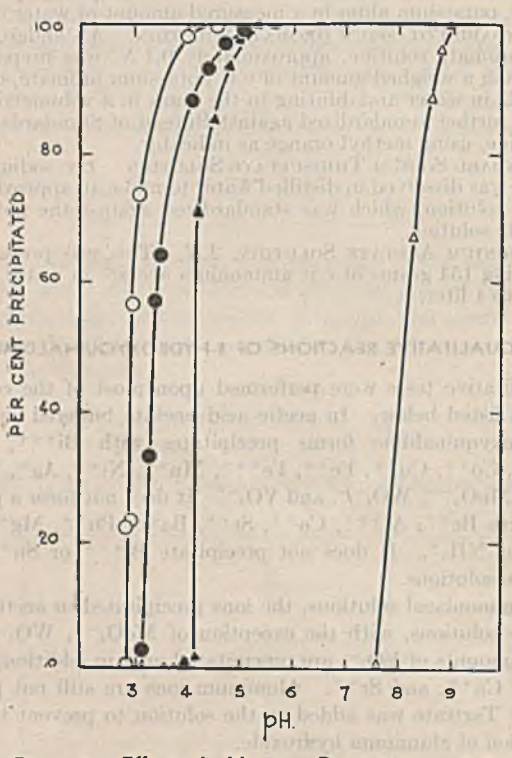

Figure 1. Effect of pH upon Precipitation of 8-Hydroxyquinaldine Complexes

○ Cupric △ Magnesium
● Ferric ▲ Zinc

It is advantageous to use an alcoholic solution of 8-hydroxyquinaldine for the volumetric determination of magnesium and an alcoholic solution may be employed in the zinc determination. The reagent is more soluble in the presence of alcohol and is not coprecipitated in the alkaline solutions. No trouble is experienced in the gravimetric determinations and when using acid solutions. The coprecipitated reagent is volatile at 130° C. The magnesium and zinc salts are soluble in hot 95% alcohol and the solubility in water is undoubtedly slightly increased by the presence of alcohol; therefore only the required amount of reagent should be employed. The presence of an excess of reagent is indicated by a yellow filtrate. If the supernatant liquid is not yellow, more reagent should be added.

Procedure for Zinc. If aluminum is present add 1 gram of ammonium tartrate to the clear, slightly acid solution. Add 2 ml. of 5% 8-hydroxyquinaldine solution in 2 *N* acetic acid for every 10 mg. of zinc present, dilute the solution to about 200 ml., and heat to 60° to 80° C. Neutralize the excess acid by adding dilute (1 to 5) ammonium hydroxide drop by drop until the zinc complex salt which forms on the addition of each drop just redissolves on stirring. Add 45 ml. of 2 *N* ammonium acetate slowly and with stirring. The pH should be at least 5.5. Allow the solution to stand for 10 to 20 minutes before filtering through a Gooch or filtering crucible if the precipitate is to be weighed or through a filter paper if the precipitate is to be determined volumetrically. If the amount of zinc is low and the amount of aluminum and magnesium is high, allow the solution to stand several hours before filtering. Wash well with hot water. If the precipitate is to be weighed, dry it at 130° to 140° C. for at least 2 hours.

Table I. Determination of Zinc, Magnesium, and Aluminum

Zn Taken *Gram*	Zn Found *Gram*	Mg Taken *Gram*	Mg Found *Gram*	Al Taken *Gram*	Al Found *Gram*
		Gravimetric results			
0.0512	0.0515	....	....	0.0500	0.0500
0.0512	0.0512	....	....	0.0500	0.0500
0.0510	0.0513	....	....	0.0100	0.0100
0.0510	0.0509	....	....	0.0100	0.0103
0.0510	0.0513	0.0287	0.0285	....	....
0.0510	0.0513	0.0115	0.0112	....	....
0.0205	0.0202	0.0115	....	0.0248	....
0.0109	0.0103	0.0115	....	0.0248	....
0.0020	0.0018	0.0287	....	0.0250	....
		Volumetric results			
0.0512	0.0510	0.0256	0.0256	....	....
0.0512	0.0516	0.0287	0.0286	....	....
0.0205	0.0208	0.0256	0.0254	....	....
0.0040	0.0044	0.0287	0.0286	....	....
0.0040	0.0042	0.0287	0.0286	....	....
0.0510	0.0518	0.0287	....	0.0250	....
0.0510	0.0507	0.0287	....	0.0250	....
0.0206	0.0204	0.0287	....	0.0250	....
0.0010	0.0013	0.0287	....	0.0100	....
0.0010	0.0013	0.0287	....	0.0100	....

To determine the zinc volumetrically, dissolve the washed precipitate with 30 ml. of hot 1 to 2 hydrochloric acid and wash thoroughly with hot 1 to 3 hydrochloric acid and then with hot water. Moisten the paper with a few drops of concentrated hydrochloric acid before the final two washings with water in order to ensure the complete solution and removal of all zinc complex salt. If the amount of zinc is small and the amount of aluminum and magnesium is large, reprecipitate the zinc as described above. Use only 1 to 2 ml. of the 8-hydroxyquinaldine reagent for the reprecipitation.

Dissolve the second precipitate in 30 ml. of hot 1 to 3 hydrochloric acid, wash the paper thoroughly with hydrochloric acid and hot water as before, and add 3 grams of potassium bromide to the filtrate. Dilute the solution to about 150 ml. and add a few drops of methyl red indicator. Run in standard 0.1 *N* potassium bromate solution from a buret until there is an excess present as shown by the bleaching of the indicator. Add 5 ml. of bromate solution in excess. Add 3 grams of potassium iodide, stir until dissolved, and back-titrate with standard sodium thiosulfate solution using a 2% starch solution as indicator.

If no aluminum is present and no tartrate has been added, the proper pH for the precipitation of zinc can be attained by adding dilute ammonium hydroxide until a white precipitate of zinc hydroxide appears. Redissolve the zinc hydroxide with a drop of acetic acid. Add 2 ml. of the acetic acid solution of 8-hydroxyquinaldine for each 10 mg. of zinc present and then 2 to 3 drops of concentrated ammonium hydroxide. The pH should be at least 5.5. This procedure eliminates the high concentration of ammonium salts and makes it easier to reach the required pH for the precipitation of magnesium. If ammonium acetate is used, the least amount possible should be added.

Procedure for Magnesium. If aluminum was not present and tartrates were not added when zinc was precipitated, the filtrate from the zinc determination can be used for the determination of magnesium. Add 3 ml. of acetic acid solution of 8-hydroxyquinaldine for every 10 mg. of magnesium present (if the determination is to be carried out volumetrically, use an alcoholic solution of the reagent) and add concentrated ammonium hydroxide until the pH is at least 9.3 or until no further precipitate forms. Digest the solution at 60° to 80° C. for 20 minutes and filter through a Gooch or filtering crucible if the magnesium is to be determined gravimetrically or through a paper filter if the determination is to be completed volumetrically. Wash with hot water, dry the precipitate at 130° to 140° C., and weigh for a gravimetric determination.

The precipitate may be dissolved in hydrochloric acid and titrated with potassium bromate according to the procedure outlined above for the volumetric determination of zinc.

Procedure for Aluminum. After the zinc has been removed, aluminum may be precipitated from the filtrate by adding 8-

hydroxyquinoline. Warm the filtrate to 60° to 80° C. and add 40 ml. of a 2.5% solution of 8-hydroxyquinoline in 7.5% acetic acid and then 10 ml. of 2 *N* ammonium acetate. Allow the precipitate to digest 10 to 20 minutes and filter through a Gooch or filtering crucible. Wash with hot water and dry at 130° to 140° C. for at least 2 hours. The precipitate may also be determined volumetrically as described above for zinc.

Results of several typical analyses are given in Table I.

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FROM a thesis submitted by Jack K. Walker to the faculty of the Graduate School in partial fulfillment of the requirements for the degree of master of science in the Department of Chemistry, Indiana University.

Determining Phytin Phosphorus

Stoichiometric Relation of Iron and Phosphorus in Ferric Phytate

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A stoichiometric relationship between phosphorus and iron in ferric phytate with an atomic ratio of 6P/4Fe was found to exist when phytic acid was precipitated with a sufficient excess of ferric chloride in the presence of sodium sulfate. On the basis of this finding, a method was developed for the determination of phytin phosphorus in corn grain.

THE determination of phytin phosphorus by the method of Heubner and Stadler (*4*) is based on the titration of phytic acid with standard ferric chloride solution with the formation of ferric phytate, in the presence of ammonium thiocyanate indicator. The titration is carried out in the presence of 0.6% hydrochloric acid. The end point, shown by the reddish brown ferric thiocyanate, is indefinite and is taken arbitrarily as the point at which the color persists for 5 minutes. It is evident that the nature of ferric phytate and particularly the ratio of iron to phosphorus in it are of vital importance in the evaluation and use of this method.

The gravimetric ratio of phosphorus to iron in Heubner and Stadler's method is 1.19. This factor has been confirmed by Rather (*5*). By using Starkenstein's (*6*) and Anderson's (*1*, *2*) formulas for phytic acid, corresponding to $C_6H_6O_6[PO(OH)_2]_6 \cdot 3H_2O$ and $C_6H_6O_6[PO(OH)_2]_6$, respectively, it may be calculated that the factor, 1.19, represents the addition of 2.8 moles of iron to 1 mole of phytic acid—that is, the gravimetric ratio of 6P/2.8Fe is 1.19. From these formulas it may also be observed that there are 12 hydrogen atoms which theoretically may be replaced by 4 moles of ferric iron. This means, therefore, that the Heubner-Stadler end point does not represent complete saturation of the phytic acid with ferric iron, but that it is rather an intermediate point in the saturation process which is reproducible with a fair degree of accuracy.

The idea of completely saturating phytic acid with iron occurred to the writer as a possible method of determining phytin phosphorus. The data from this investigation, presented in this paper, indicate that 1 mole of phytic acid (inositol hexaphosphoric acid) under proper conditions will add 4 moles of ferric iron.

In this case the relationship of phosphorus to iron in tetraferric phytate is 6 moles of the former to 4 of the latter. This atomic ratio, 6P/4Fe, corresponds to the gravimetric factor, 0.833. This relation places iron and phosphorus on a chemical equivalent basis in the molecule and obviates the necessity of using an empirical factor. The empirical formula of this compound is believed to be $C_6H_6O_{24}P_6Fe_4 \cdot 3H_2O$. However, in view of the polyvalence of both phytate and ferric ions, it is unlikely that any such molecules as above formulated are formed. The probability is very great that the respective ions unite in positions that may be termed "out of phase", with the result that a polymeric type of precipitate is formed with no definite molecular boundaries. The gradual rather than stepwise loss of reactivity with the decrease of replaceable hydrogen on approaching the Heubner and Stadler end point, points to this conclusion as well as the absence of a definite end point. This conclusion is also supported by the nonintegral atomic ratio of 6P/2.8Fe in the precipitate formed at the end point chosen by Heubner and Stadler.

In studying the relationship of phosphorus and iron in ferric phytate, two sources of phytic acid were used—namely, calcium phytate and corn grain extract.

The calcium phytate was obtained from the Soil Biology Department. It was shaken with a large excess of distilled water to dissolve any soluble fractions which might be present. It was then filtered, washed with additional water, then alcohol, and dried at 100° C. for 30 hours. This substance contained 16.09% total phosphorus and from its reaction with iron corresponded to the formula $C_6H_6O_{24}P_6Ca_6 \cdot 3H_2O + 12H_2O$. The data in Table I were secured with this material according to the procedure given for the corn grain extract.

The remainder of the data in the paper were secured on phytic acid freshly extracted from corn grain. The freshly ground corn was extracted with 1.2% hydrochloric acid, containing 10% by weight of sodium sulfate, for 2 hours on the shaking machine. The ratio of grain to solvent was 1 gram to 20 ml. The acid ex-

Table I. Phosphorus-Iron Ratios in Ferric Phytate Precipitate

(As influenced by iron-phosphorus ratio in the precipitating mixture. Precipitation from acidified calcium phytate)

In Precipitating Mixture			In Precipitate		
P, Mg.	Fe, Mg.	Multiple of theoretical ratio, 4Fe/6P	Fe, Mg.	P/Fe	Atomic ratio
				1.19[a]	6P:2.80Fe[a]
4.18	8.05	1.6	4.18	1.000	6P:3.33Fe
4.18	10.04	2.0	4.54	0.921	6P:3.62Fe
4.18	10.04	2.0	4.33	0.965	6P:3.45Fe
4.18	12.05	2.4	4.78	0.874	6P:3.81Fe
4.18	12.05	2.4	4.67	0.895	6P:3.72Fe
4.18	17.31	3.4	4.99	0.838	6P:3.98Fe
4.18	17.31	3.4	4.77	0.876	6P:3.80Fe
4.18	43.27	8.6	5.02	0.833	6P:4.00Fe

[a] Values which correspond with Heubner-Stadler end point; not experimental.

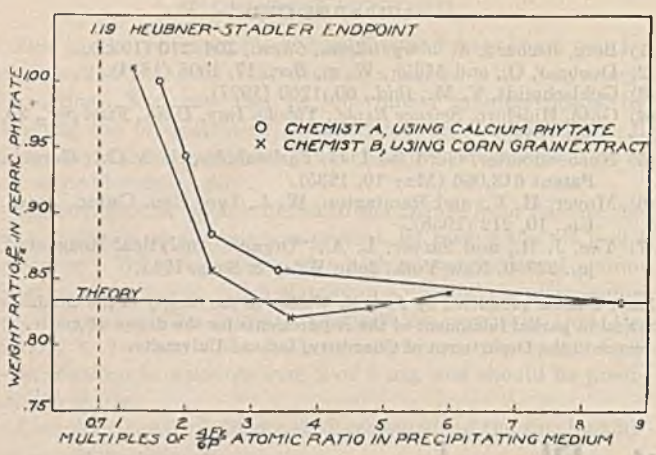

Figure 1. Relation of Concentration of Iron in Precipitating Medium to Composition of Ferric Phytate Precipitate

tract was centrifuged and filtered through a filter paper and asbestos using suction. About 800 ml. of corn extract were prepared. Phytic acid from several aliquots of this stock solution was precipitated with excess iron. The precipitate was filtered on an asbestos mat in a Gooch crucible with suction and washed 5 times with 0.3% hydrochloric acid–2.5% sodium sulfate solution. The precipitate was moistened with a few drops of 50% magnesium nitrate solution and ignited in a muffle for 1 hour at about 1000° C. The asbestos mat and residue were transferred into a 250-ml. beaker and the residue was dissolved in about 10 to 15 ml. of 1 to 1 hydrochloric acid. The solution was filtered and made to 200 ml. with distilled water, and total phosphorus was determined (*3*). The total milligrams of phosphorus found in the iron precipitate represented the phytin phosphorus (*7*) contained in the aliquot taken from the stock solution.

The relationship of phosphorus and iron in ferric phytate was obtained by precipitating the phytic acid in aliquots of these stock solutions with a known excess of standard ferric chloride solution for about 1 hour. The ferric chloride solution contained about 0.2% iron and 0.6% hydrochloric acid (*4*). The total volume also contained 0.6% hydrochloric acid (*4*) and about 4% sodium sulfate. Excess iron in the supernatant liquid was determined as described below, and from these data the ratio of phosphorus to iron in ferric phytate was calculated.

EFFECT OF IRON CONCENTRATION IN PRECIPITATING MIXTURE ON PHOSPHORUS-IRON RATIO IN FERRIC PHYTATE

The quantity of iron which exists in combination with a given quantity of phytic acid should remain constant over a reasonable range of iron concentration after complete saturation has taken place, if a stoichiometric relation exists between phosphorus and iron in ferric phytate with an atomic ratio of 6P/4Fe. Prior to complete saturation of the phytic acid the quantity of iron reacting with phytic acid should increase with increasing concentration of iron in the precipitating solution.

Experimental data concerning this reaction are given in Tables I and II and Figure 1. Table I represents one of the earlier experiments with calcium phytate in which the duplicates do not agree so closely as in the later work with corn extract. These data indicate that in the precipitation of ferric phytate the extent to which the 12 hydrogen atoms in the phytic acid are replaced by iron is determined by the ratio of iron to phosphorus in the precipitating mixture in the range below about 3.6 times the theoretical ratio in the ferric phytate precipitate—i.e., 4 atoms of iron to 6 of phosphorus. At about this ratio (3.6 × 4Fe/6P) the hydrogens of the phytic acid are all replaced by iron, with the result that the presence of any larger ratio of iron to phosphorus has no effect upon the composition of the precipitate although it may accelerate its formation.

Nearly all of the first 3 moles of iron are added to the phytic acid with ease. This is indicated by the fact that in the Heubner and Stadler titration the phytic acid removes all the iron from the solution almost instantly until their end point, corresponding to a 2.8-mole addition, is almost reached. The addition of the fourth mole of iron to the phytic acid proceeds with increasing difficulty, as is evidenced by the necessity of more than trebling the theoretical atomic Fe/P ratio in the precipitating medium in addition to allowing considerable time for the reaction to go to completion.

Complete iron saturation of the phytic acid, with an atomic ratio of 6P/4Fe, corresponds to a gravimetric ratio, P/Fe-0.833. This is essentially the ratio obtained in the precipitate where sufficient iron was used and adequate time allowed for the reaction.

COMPOSITION OF FERRIC PHYTATE PRECIPITATE

In addition to the values obtained by analysis of the residual iron in the supernatant solution, an experiment was conducted in which both the supernatant solution and the ferric phytate precipitate were analyzed for iron and phosphorus. As will be seen in the last two columns of Table III, the mean composition of the ferric phytate precipitate as calculated from the analysis of the supernatant solution gives a gravimetric P/Fe ratio of 0.836, which is in close agreement with the theoretical value of 0.833.

On the other hand, direct analyses of the ferric phytate precipitate gave values for iron somewhat higher than the theoretical, resulting in ratios similar to those reported by Wrenshall and Dyer (*7*), whose paper appeared after this research was completed. It is believed that the high iron values are a result of the difficulty of washing the ferric phytate precipitate free of inorganic iron and that therefore the analysis of the supernatant solution is the technique which should be followed.

QUANTITATIVE METHOD FOR PHYTIN PHOSPHORUS

On the basis of the results of this investigation, a method for determining phytin phosphorus in corn grain has been developed.

Table II. Phosphorus-Iron Ratios in Ferric Phytate Precipitate

(As influenced by iron-phosphorus ratio in precipitating mixture. Precipitation from corn grain extract)

In Precipitating Mixture		Multiple of theoretical	In Precipitate		
P, Mg.	Fe, Mg.	ratio, 4Fe/6P	Fe, Mg.	P/Fe	Atomic ratio
3.31	4.75	1.2	3.28	1.009	6P:3.30Fe
3.31	4.75	1.2	3.23	1.025	6P:3.25Fe
3.31	9.50	2.4	3.89	0.851	6P:3.92Fe
3.31	9.50	2.4	3.84	0.862	6P:3.87Fe
3.31	14.25	3.6	4.04	0.819	6P:4.07Fe
3.31	14.25	3.6	4.02	0.823	6P:4.05Fe
3.31	19.00	4.8	4.00	0.828	6P:4.02Fe
3.31	19.00	4.8	4.00	0.828	6P:4.02Fe
3.31	23.75	6.0	3.94	0.840	6P:3.97Fe
3.31	23.75	6.0	3.94	0.840	6P:3.97Fe

Table III. Relation of Phosphorus and Iron in Ferric Phytate Precipitate as Determined by Two Procedures

Aliquot	P in Precipitate (Colorimetrically)	Multiple of Theoretical Ratio, 4Fe/6P[a]	Fe in Precipitate		P/Fe in Precipitate	
	Mg.		Mg.[b]	Mg.[c]	Mg.[b]	Mg.[c]
A	3.30	2.4	3.89	4.65	0.848	0.710
	3.30	2.4	3.84	4.70	0.859	0.702
B	3.30	3.6	4.04	4.20	0.817	0.786
	3.30	3.6	4.02	4.20	0.821	0.786
C	3.33	4.8	4.00	4.40	0.833	0.757
	3.33	4.8	4.00	4.35	0.833	0.765
D	3.31	6.0	3.94	4.30	0.840	0.770
	3.31	6.0	3.94	4.60	0.840	0.720
				Mean	0.836	0.75
				Theory	0.833	

[a] In precipitating mixture.
[b] By analysis of supernatant solution.
[c] By analysis of ferric phytate precipitate.

Table IV. Comparison of Phytin Phosphorus Values

As determined by proposed iron method and by analyses of ferric phytate precipitate)

Corn Sample	Weight of Sample	In Precipitate: Fe[a]	In Precipitate: Phytin P (Fe × 0.833)	In Precipitate: Phytin P[b]	In Corn Grain: Total P	In Corn Grain: Phytin P	In Corn Grain: Phytin P of total P
	Grams	*Mg.*[c]	*Mg.*	*Mg.*[c]	%	%	%
46	1.2249	4.05	3.37	3.34	0.312	0.275	88.1
47	1.1948	3.50	2.91	2.88	0.281	0.243	86.5
83	1.1956	3.55	2.96	2.96	0.285	0.247	86.7
86	1.2228	3.99	3.32	3.30	0.312	0.272	87.2
87	1.1901	3.82	3.18	3.24	0.299	0.267	89.3
90	1.1791	3.65	3.44	3.10	0.296	0.258	87.2

[a] Determined by analysis of supernatant solution.
[b] Determined by analysis of ferric phytate precipitate.
[c] Average of duplicate determinations from acid extract of a single sample.

Weigh 4.6000 grams of finely ground corn grain into a 200-ml. Erlenmeyer flask, add exactly 100 ml. of 1.2% hydrochloric acid (5) containing 10% sodium sulfate by weight, and shake on a mechanical shaker for 2 hours. Decant the supernatant liquid into a centrifuge tube and centrifuge for about 10 minutes. Decant the liquid through a dry filter paper into a dry beaker. Immediately pipet 50 ml. of the extract into a clean, dry 200-ml. Erlenmeyer flask, add 50 ml. of distilled water, and mix thoroughly. Add 15 ml. of standardized ferric chloride solution prepared in 0.6% hydrochloric acid and containing approximately 0.2% iron. Rotate the flask gently while adding the iron solution. Stopper the flask and continue to rotate until the ferric phytate forms. Then let stand about 1 hour with occasional shaking. Decant the solution through a dry filter paper into a clean, dry beaker or centrifuge the solution. Immediately pipet 50 ml. of this solution into a 100-ml. beaker, bring hydrochloric acid concentration up to 1 *N*, and put through a Walden silver reductor in two portions. Rinse the beaker with six 25-ml. portions of 1 *N* hydrochloric acid, allowing each portion to drain almost to the top of the silver column before adding the next. Catch the solution and washings in a 500-ml. Erlenmeyer flask, add about 0.25 gram of sodium fluoride and 3 drops of sodium diphenylamine sulfonate indicator, and titrate the ferrous iron immediately with 0.00895 *N* potassium dichromate (1 ml. = 0.5 mg. of iron). The end point is very sharp, changing from slightly yellow to purple.

From the titration value the milligrams of inorganic iron in the 50-ml. aliquot may be ascertained, and the value, subtracted from the total milligrams of iron originally added to the aliquot, gives the milligrams of iron chemically bound as ferric phytate. This latter quantity multiplied by 0.833 gives milligrams of phytin phosphorus in 1.0 gram of grain.

This method was further checked on samples of corn grain by comparing the phytin phosphorus obtained by the proposed iron method with that obtained from the ferric phytate precipitate. The iron in the precipitating mixture ranged from 3.1 to 3.6 times the theoretical amount required to react completely with the phytic acid (Table IV).

The results of this test indicate that phytin phosphorus in corn grain may be determined as accurately by the proposed iron method as by the determination of phytin phosphorus in the ferric phytate precipitate.

ACKNOWLEDGMENT

The author wishes to thank E. E. DeTurk, professor of soil fertility, for his assistance in preparing this paper, and K. M. Peng, assistant in soil fertility, for his help with the chemical analyses.

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Determination of Small Amounts of Sulfate in Cellulose Nitrate and Other Cellulose Esters

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THE relationship between sulfate content and stability of cellulose nitrate has long been recognized (4). In the process of stabilization of cellulose nitrate the sulfate content approaches zero and the determination of sulfate becomes increasingly difficult.

A number of methods for estimating sulfate in cellulose esters have been described. Cross, Bevan, and Briggs (2) used aqua regia to decompose the sample and determined sulfate gravimetrically on the digest. Berl and Bemmann (1) and Hake and Lewis (4) decomposed the organic material with alkali. Kullgren (5) decomposed the sample with hydrochloric acid, evaporated the solution to dryness, burned the residue in a combustion tube in a current of oxygen, and absorbed the evolved sulfuric acid in sodium hydroxide solution. Dunnicliff (3) oxidized the cellulose nitrate with nitric acid and sodium chlorate and determined the sulfate gravimetrically. Malm and Tanghe (6) decomposed cellulose acetate by refluxing with nitric acid, completed the oxidation with potassium nitrate, and determined sulfate gravimetrically after removing nitrate by evaporation with hydrochloric acid. When the sulfate content of cellulose nitrate or other esters is very low these methods require the use of large samples to provide sufficient amounts of the barium sulfate precipitate for convenient manipulation and the decomposition becomes lengthy and tedious.

A method of analysis which has proved convenient and which gives reproducible results involves decomposition of the cellulose nitrate with nitric acid to which a small amount of perchloric acid is added after the initial stage of digestion. The sulfate in the digest is determined by a modification of the Morgulis and Hemphill (7) method. Barium chromate dissolved in dilute hydrochloric acid reacts with sulfate ions to give a precipitate of barium sulfate and an equivalent amount of chromic acid which can be determined iodometrically after the excess barium chromate is precipitated by making the solution alkaline with ammonia. This procedure determines total sulfur, but it is assumed that practically all the sulfur in cellulose nitrate is in the form of sulfate. This assumption is implicit in almost all methods for determining sulfate in cellulose nitrate and seems reasonable in view of the processes and materials used in its manufacture.

REAGENTS

BARIUM CHROMATE REAGENT. Prepare pure barium chromate by double decomposition, using solutions containing theoretical amounts of barium chloride and potassium dichromate. Wash the barium chromate thoroughly with 1% acetic acid and then with water and dry. Dissolve 2.53 grams of barium chromate in 100 ml. of 2 *N* hydrochloric acid and dilute to 1 liter.

POTASSIUM IODATE, 0.01 *N*. Dissolve 0.3567 gram of pure potassium iodate in water and dilute to exactly 1 liter.

SODIUM THIOSULFATE SOLUTION, 0.002 *N*. Standardize against the standard potassium iodate solution at the same time the determinations are titrated.

PERCHLORIC ACID, 60%.
NITRIC ACID, concentrated reagent grade.
STARCH INDICATOR. Dissolve 1 gram of soluble starch (*8*) in 100 ml. of boiling water.
AMMONIUM HYDROXIDE, concentrated reagent grade.
SULFURIC ACID, 10%.
POTASSIUM IODIDE, crystals which give no test for free iodine.

METHOD

Weigh accurately into a 50-ml. beaker a sample of cellulose nitrate containing between 0.4 and 1.2 mg. of sulfate. The sample should not exceed about 2 grams. Place a small stirring rod in the beaker, add 20 ml. of concentrated nitric acid, cover with a watch glass, and heat on a steam bath until the cellulose nitrate dissolves. Add 3 ml. of 60% perchloric acid and heat on a hot plate so that the solution boils gently. Digest until copious white fumes are evolved. If the solution is colored, add about 10 ml. of water and again digest until white fumes appear. Continue the digestion until the volume of solution in the beaker is less than 1 ml. Do not allow the solution to approach dryness, since this leads to low values and may introduce a hazard. Transfer to a 15-ml. graduated centrifuge tube. Wash the beaker thoroughly by using a medicine dropper, being careful that the total volume does not exceed 5 or 6 ml. Add 5 ml. of barium chromate reagent and allow precipitate to form for at least 4 hours or preferably overnight. Make alkaline with concentrated ammonium hydroxide (about 3 ml.). Add water to make the total volume exactly 15 ml., mix well, allow to stand for 1 hour, and centrifuge.

Pipet a 5-ml. aliquot of the supernatant liquid into a 50-ml. Erlenmeyer flask. Add approximately 50 mg. of potassium iodide and 8 drops of starch indicator. Acidify with 10% sulfuric acid and titrate with sodium thiosulfate solution to disappearance of starch iodide color.

If more than 0.4 mg. but less than 1.2 mg. of sulfate is present in the sample, the sulfate content may be calculated from the stoichiometric factor. One milliliter of 0.002 *N* sodium thiosulfate is equivalent to 0.064 mg. of sulfate (SO_4) or 0.021 mg. of sulfur. If there is less than 0.4 mg. of sulfate in the sample, repeat the determination, using a larger sample, since titrations in this range vary with the amount of perchloric acid remaining after digestion.

DISCUSSION AND EXPERIMENTAL

Certain details in the procedure have been incorporated to reduce danger of explosions. If perchloric acid which is both hot and concentrated is brought into contact with organic material, a definite explosive hazard exists. By first degrading the cellulose nitrate with concentrated nitric acid and then adding a small amount of perchloric acid this danger is eliminated because the perchloric acid is diluted by the nitric acid. As the solution evaporates, the organic material is oxidized, so that before the perchloric acid has become concentrated the organic material is decomposed. The solution is never allowed to approach dryness. In the course of several hundred determinations by this method no explosions have occurred.

Table I. Sulfate Recovery Data

Sample (1 Gram)	Sulfate Added [a] Mg.	Sulfate Found Mg.	Sulfate Calculated Mg.
Cellulose nitrate	0.00	1.27	..
		1.25	
	0.19	1.47	
		1.45	1.45
Cellulose nitrate	0.00	0.48	..
	0.48	0.95	
		0.95	0.96
Cellulose acetate	0.00	0.31	..
		0.31	
	0.48	0.77	
		0.78	0.79
Glucose pentaacetate	0.00	0.00	..
		0.00	
	0.48	0.49	
		0.50	0.48
Dextrose	0.00	0.00	..
		0.00	
	0.48	0.49	..
		0.50	0.48
Cellulose acetate propionate	0.00	0.27	..
		0.27	
	0.48	0.74	
		0.75	0.75
Cellulose acetate butyrate	0.00	0.09	..
		0.10	
	0.48	0.58	
		0.59	0.58

[a] Added as 0.001 *M* H_2SO_4.

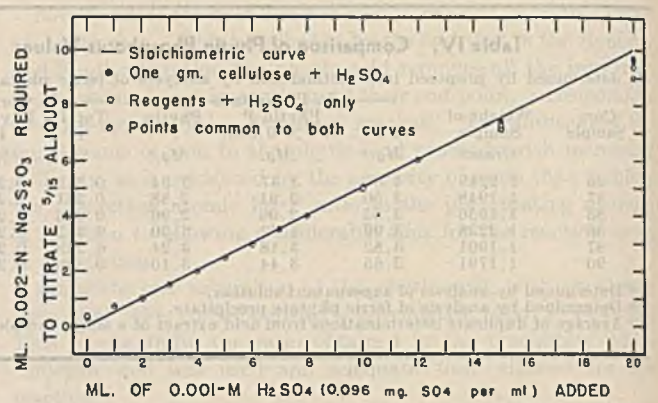

Figure 1. Recovery of Sulfate in the Presence and Absence of Cellulose

The range and accuracy of the method were established by weighing a series of 1-gram samples of sulfate-free cotton cellulose into beakers, to which were added definite amounts of a 0.001 *M* (0.096 mg. of SO_4 per ml.) solution of sulfuric acid. The determinations were then carried out as outlined above. A similar series omitting the purified cellulose was also treated in the same manner. In Figure 1 the values obtained by titration of a 5/15 aliquot with 0.002 *N* sodium thiosulfate are plotted against the milliliters of 0.001 *M* sulfuric acid added to the sample. The stoichiometric curve is drawn on the same figure. When there is less than about 0.4 mg. of sulfate in the sample, the titration values are not reproducible and are usually considerably above theory. In this range the value of the titration is dependent on the amount of perchloric acid remaining after digestion. If 1 ml. or more of perchloric acid is allowed to remain after digestion, titration values considerably greater than those shown in in Figure 1 for 0 to 4 ml. of 0.001 *M* sulfuric acid may be obtained. If more than 1.2 mg. of sulfate is present, the titration values tend to be low. Within the range of 0.4 to 1.2 mg. of sulfate the points fall on the stoichiometric curve, so that no deduction for a blank is required for the reagents which were used. This fact should be established for each set of the reagents prepared.

In order to check the accuracy of the method, samples of cellulose nitrate and other related organic materials were analyzed both with and without the addition of known amounts of sulfuric acid. The results, shown in Table I, indicate that sulfate in cellulose esters may be accurately determined by the method. Any ion such as phosphate which forms an insoluble barium salt would interfere. None of these was present in the materials analyzed.

ACKNOWLEDGMENT

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Photoelectric Automatic Liquid Level Control

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An automatic level control consisting of a photoelectric relay operating a solenoid valve is described. The beam of light actuating the relay is directed at an angle onto the photocell so that the water level, on rising, refracts the beam away from the photocell and closes the solenoid valve.

MANUALLY maintaining a constant liquid level in a forced-circulation vacuum evaporator that had been set up in the laboratory was found difficult. A number of methods of providing automatic level control were considered.

The choice of the type of control to be used was influenced by the following conditions:

The evaporator is operated under a considerable vacuum and the control-actuating device, therefore, must not introduce air leaks into the system.

The level to be controlled is considerably removed from the point at which feedwater is introduced into the system.

The surface of the water is in constant agitation because of the forced circulation and boiling.

It was decided that an on-off control would be desirable because of its simplicity and positive action, its inherent cycling character being no disadvantage since moderate fluctuations in level have no effect on operation of the evaporator.

The control which was installed is adapted to operation under the above conditions and consists basically of a photoelectric relay which is controlled by the rise and fall of the level of the water in the evaporator. The relay in turn operates a solenoid valve which controls the feedwater inlet. Sight glasses had already been built into the evaporator and no further vacuum-tight fittings had to be installed.

The water level was made to control the light beam despite its transparency by having the light beam at an angle with the horizontal, so that the beam was refracted away from the photocell by the rising water. A time delay was incorporated into the action of the relay, so that rapid changes in the light beam resulting from continuous agitation of the surface would not affect the relay.

The fundamental circuit of an alternating current–operated phototube relay is shown in Figure 1 (*1*).

The circuit is that of an ordinary amplifier with the relay as its load. The tube acts as its own rectifier, condenser C_1 preventing the relay from chattering at 60 cycles. Potentiometer P supplies grid bias for the tube and, with the photocell dark, is adjusted so that the relay has just insufficient strength to close the contacts. When the phototube is illuminated it passes more current and makes the grid less negative, thus increasing the plate current and operating the relay. When the illumination is removed, the relay again opens. Condenser C_2 serves to eliminate the phase difference between grid and plate voltages.

The complete circuit diagram of the photoelectric relay as installed is shown in Figure 2.

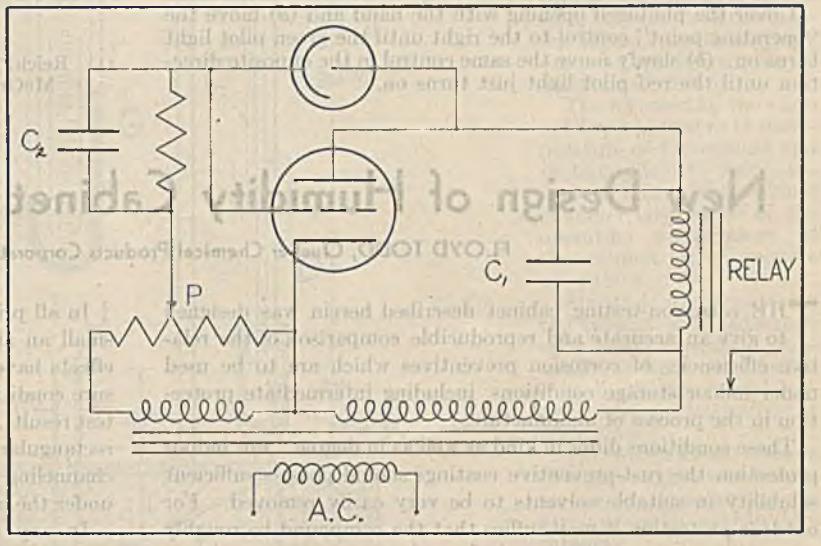

Figure 1. Alternating Current–Operated Phototube Circuit

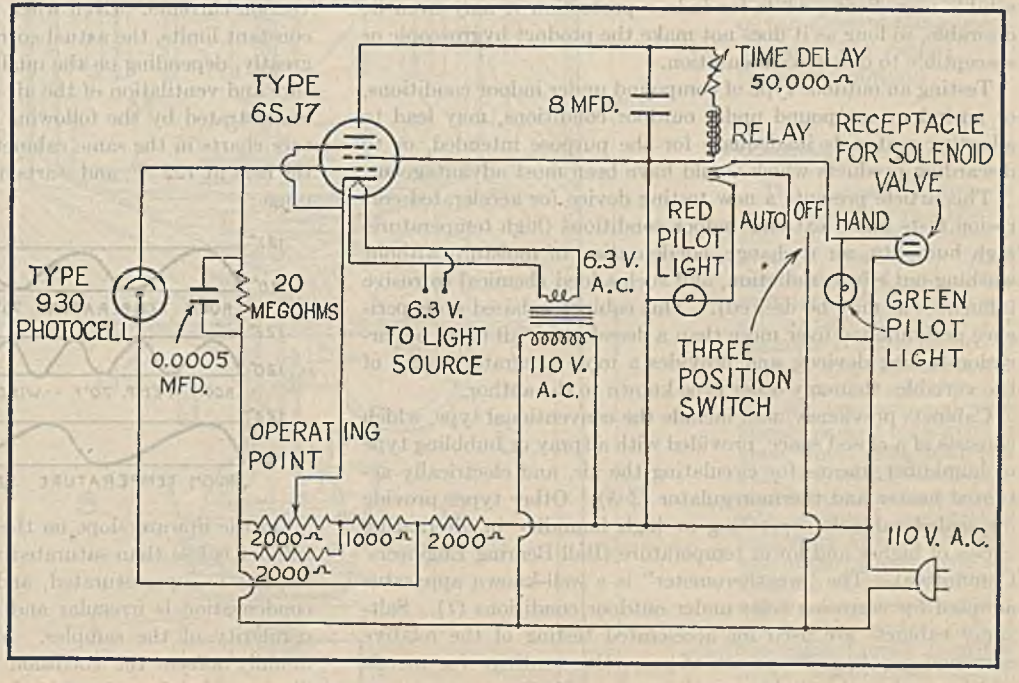

Figure 2. Complete Circuit Diagram

[1] Present address, Midwest Consultants, St. Louis, Mo.

The special transformer appearing in the circuit of Figure 1 is replaced by a voltage-divider arrangement supplying grid bias and the reduced voltage necessary to prevent the gas-filled phototube from glowing. The Type 6SJ7 amplifier tube has suitable plate current to operate the type of relay which was obtained. Time delay is introduced by the inductive-resistive effect of a variable resistor in series with the relay. A three-position switch provides for automatic or hand operation and the two pilot lights indicate whether the relay is open or closed (red indicates valve closed and green valve open). The relay and photocell are wired into the circuit in such a way that the valve will be closed during the period in which the tube is warming up, and also under such extraordinary circumstances as failure of the light source, sudden ebullition because of increased vacuum, etc.

The light source consists of a 32-candlepower automobile headlight bulb fitted with a lens to focus an image of the filament onto the photocell. The solenoid valve was manufactured by the Minneapolis-Honeywell Regulator Company and requires 0.25-ampere steady current at 110 volts alternating current. If the feed enters at atmospheric pressure, the valve should be designed for a pressure of not more than 15 pounds per square inch. If its rated pressure is too high, the valve will not close properly.

METHOD OF OPERATION. Place the three-position switch in the automatic position and the "time delay" control at maximum.

Cover the photocell opening with the hand and (*a*) move the "operating point" control to the right until the green pilot light turns on; (*b*) slowly move the same control in the opposite direction until the red pilot light just turns on.

The adjustment is critical and should be repeated if the relay chatters or fails to open and close.

This unit has been in operation for over a year and has proved very satisfactory. Anyone with a little radio experience can easily build a control of this type at a total cost of from $35.00 to $50.00.

PARTS FOR PHOTOCELL RELAY

1 SPDT sensitive relay, 1 to 2 ma. to close, contacts rated at 5 amperes noninductive A.C.
1 Type 6SJ7 receiving tube (Type 6SJ7GT may be substituted)
1 Type 930 gas-filled phototube
1 50,000-ohm wire-wound linear potentiometer
1 2000-ohm wire-wound linear potentiometer
2 2000-ohm, 10-watt wire-wound resistors
1 1000-ohm, 10-watt wire-wound resistor
1 20-megohm, 0.5-watt carbon resistor
1 0.0005-mfd. mica condenser
1 8-mfd. 450-volt electrolytic condenser
1 transformer, 110 to 6.3 volts at 6 amperes
2 110-volt, 6-cp. pilot lights, candelabra base
1 6- to 8-volt, 32-cp. Mazda bulb, No. 1132
1 SPDT toggle switch, center off position
1 2-pole round Bakelite receptacle

LITERATURE CITED

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New Design of Humidity Cabinet for Corrosion Testing

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THE corrosion-testing cabinet described herein was designed to give an accurate and reproducible comparison of the relative efficiencies of corrosion preventives which are to be used under indoor storage conditions, including intermediate protection in the process of manufacture.

These conditions differ in kind as well as in degree. For indoor protection the rust-preventive coatings should possess sufficient solubility in suitable solvents to be very easily removed. For outdoor protection it may suffice that the compound be roughly removable, for example, by wiping. Furthermore, for outdoor protection any appreciable water-solubility of the protective agent is very objectionable, as rain would remove any water-soluble ingredients, while for indoor protection it may even be desirable, so long as it does not make the product hygroscopic or susceptible to dew or condensation.

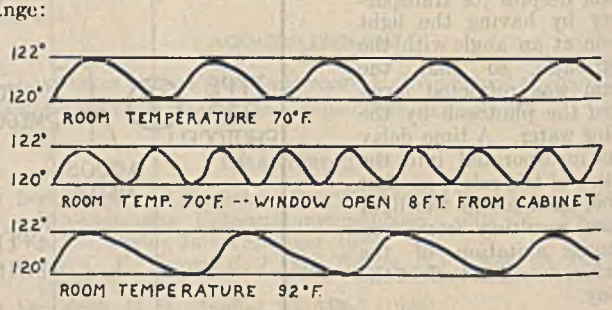
Testing an outdoor type of compound under indoor conditions, or an indoor compound under outdoor conditions, may lead to adopting materials inadequate for the purpose intended, or to discarding products which would have been most advantageous.

This article presents a new testing device for accelerated corrosion tests under extreme indoor conditions (high temperature, high humidity, air exchange, condensation of moisture without washing-out effect, radiation, and such added chemical corrosive influences as may be desired). This cabinet is based on experience accumulated over more than a decade with all types of corrosion-testing devices, and provides a more accurate control of the variables than any other type known to the author.

Cabinets previously used include the conventional type, which consists of a closed space, provided with a spray or bubbling type of humidifier, means for circulating the air, and electrically actuated heater and thermoregulator (*4–8*). Other types provide for sealed cabinets, operating at high humidity in alternating cycles of higher and lower temperature (Ball Bearing Engineers' Committee). The "weatherometer" is a well-known apparatus adopted for corrosion tests under outdoor conditions (*1*). Salt-spray cabinets are used for accelerated testing of the relative corrosion proofing efficiencies of protective coatings for metals which may be subjected to sea air exposure (*2, 3*).

In all prior apparatus the heating has been centralized in too small an area. As a result, convection currents and radiation effects have occurred within the cabinets, causing uneven exposure conditions and consequent serious irregularities in corrosion test results. Furthermore, many humidity cabinets are square or rectangular in shape. Such a design is conducive to undesirable channeling of air and moisture in those cabinets which operate under the usual dynamic exposure conditions.

In any thermostatically controlled apparatus, whether humidity or salt-spray cabinet, the temperature fluctuates within a range of 1° to 3° F. (0.5556° to 1.6668° C.), because of the lag in the regulator. These variations may be accentuated by convection currents. Even when the temperature fluctuates within constant limits, the actual corrosion temperature ratio may vary greatly, depending on the quality of insulation and the temperature and ventilation of the air surrounding the apparatus. This is illustrated by the following curves, which show the temperature charts in the same cabinet, in which the regulator shuts off the heat at 122° F. and starts heating 120°, a better than average range:



On the upward slope on the temperature curve the air in the cabinet is less than saturated with moisture; on the downward slope it is supersaturated, and condensation may occur. This condensation is irregular and emphasizes any slight surface irregularity of the samples. Moreover, the type of cycle profoundly affects the corrosion behavior, leading to appreciable discrepancies in corrosion time and particularly in corrosion

types, dependent on external factors not controlled in any cabinet specifications with which the author is familiar.

A humidity cabinet for corrosion testing has therefore been designed in order to eliminate the disadvantages of prior cabinets. Its design gives additional advantages inherent in only this type of cabinet.

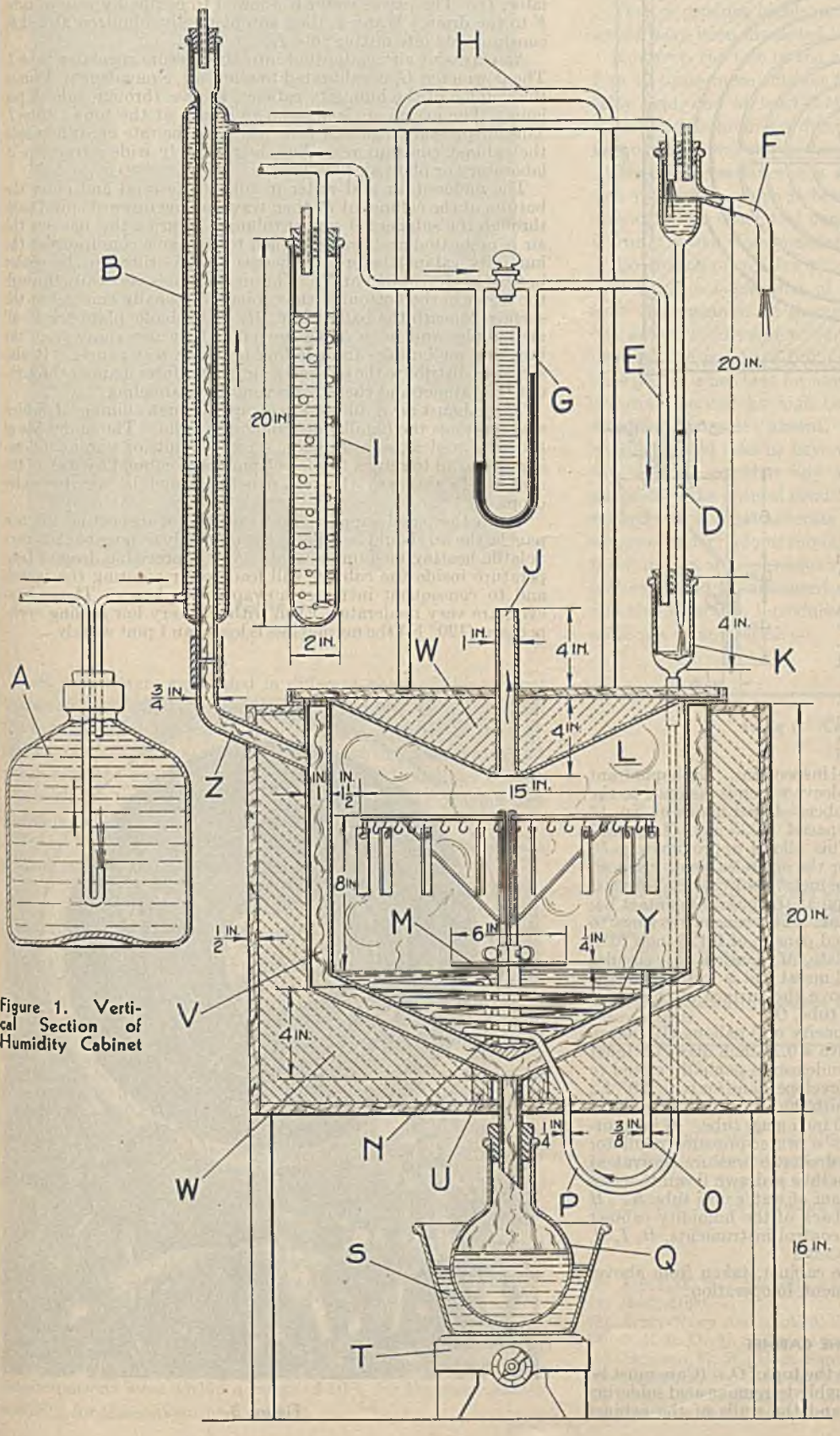


Figure 1. Vertical Section of Humidity Cabinet

The walls and bottom are heated over a preponderant area. This heating is effected by means of the vapors of a constant-boiling liquid and is therefore absolutely uniform and lagless. Convection currents are thus completely eliminated.

Still more important, the temperature "curve" is here a straight line, the cycles of recurrent sub- and supersaturations are eliminated completely, because the temperature is that of the boiling point of a constant-boiling liquid, and no mechanical regulation is necessary.

The uniformity of the temperature and humidity conditions within the cabinet give accurate comparisons of the relative efficiencies of corrosion preventives, platings, varnishes, or other protective coatings. These results can be accurately reproduced.

By using cyclopentane as the heating liquid a temperature of 120° F. (49° C.) is maintained, which is a commonly specified condition. By selecting the heating liquid, practically any temperature desired can be maintained exactly.

The air used in the cabinet is preheated to the temperature of the cabinet and prehumidified to 100% before it enters the cabinet chamber, regardless of the operating temperature of the cabinet or of outside conditions.

Air and water entering the cabinet are automatically and accurately controlled and are not affected even by relatively wide variations in the laboratory or plant lines.

The design avoids corners or sharp angles, which tend to cause irregularities in air currents within the cabinet.

The only variable, that of the effect on boiling point of variations of atmospheric pressure, has proved practically insignificant.

CONSTRUCTION OF CABINET

The cabinet, shown in vertical section in Figure 1 and in horizontal section in Figure 2, consists of a vapor-tight jacketed cylindrical container, *V*, with a conical bottom and top. The chamber is made of No. 22 B. & S. gage Monel metal. The annular spaces and lid, *W*, are insulated with loosely packed rock wool or glass wool. Fourteen feet (420 cm.) of 0.25-inch (0.6-cm.) copper tubing are bent into a spiral and tacked uniformly around the bottom of the cabinet chamber. The top of the spiral tubing is bent abruptly downward in the central part of the cabinet with its orifice at *N*. An overflow tube, *O*, is used to keep a constant

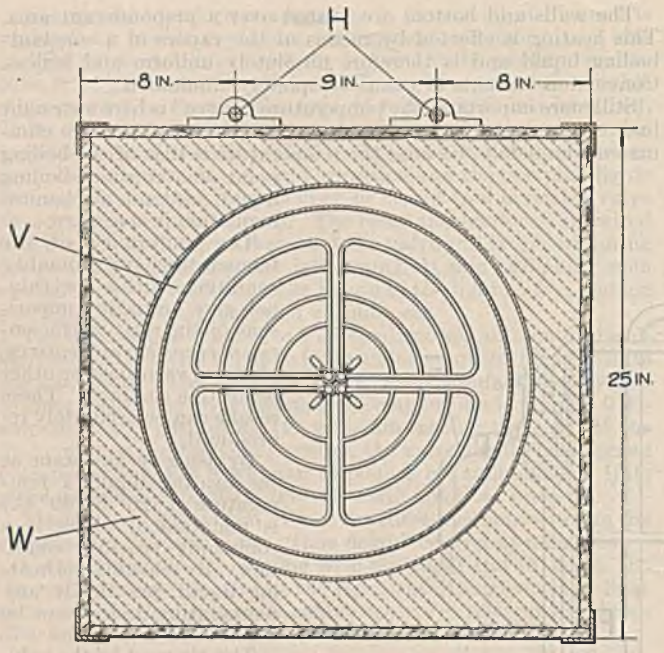
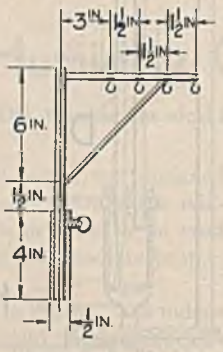


Figure 2. Horizontal Section of Cabinet



water level in the cabinet as shown by the horizontal dotted line. A wooden support, *U*, is used. The cabinet is mounted in a 0.5-inch (1.25-cm.) plywood container. The heating flask, *Q*, is a 2-liter round-bottomed Pyrex flask in an oil bath, *S*.

The test panel supports consist of 4 concentric rings which are divided into quadrants. Each quadrant is a separate unit and may be removed separately for panel inspections. Each quadrant slides into its own vertical support sleeve which is fastened to the center bottom of the cabinet chamber. Panel hooks of No. 16 B. & S. gage Monel metal are spaced 0.625 inch (1.58 cm.) apart on the concentric rings. This allows approximately 50 hooks per quadrant or 200 hooks for the entire cabinet. Experience has shown that the use of these metal hooks instead of glass hooks causes no perceptible corrosion at the point of contact between the panel and the metal hook. The concentric rings to which the panel hooks are supported consist of 0.125-inch (0.3-cm.) Monel metal rod. A baffle plate, *M*, consists of a circular piece of No. 22 B. & S. gage Monel metal and is fastened to the panel support sleeves 0.25 inch above the surface of the water level as determined by the overflow tube, *O*.

Sedimentation bottle *A* has a capacity of 5 gallons (19 liters). Reflux condenser *B* is fairly long with a 0.75-inch diameter inner condenser tube. The top of the condenser is partially closed to decrease loss of the volatile solvent (cyclopentane for b.p. 120° F., acetone for 136°, etc.) by diffusion into the air. The air regulator and safety tube, *I*, is a large 2 × 20 inch glass tube. *G* is an ordinary calibrated flowmeter. *D* is a water-pressure regulator which operates under a constant hydrostatic pressure equivalent to 22 inches. The lower end of this tube is drawn down to a fine capillary, so as to admit a fine stream of water into tube *K*. *H* is an iron support fastened to the back of the humidity cabinet container and is used to hold the control instruments, *B*, *I*, *G*, *E*, and *D*.

Figure 3 is an inside view of the cabinet, taken from above; Figure 4 shows the complete instrument, in operation.

OPERATION OF THE CABINET

The cabinet is filled with water to the top of *O*. (Care must be exercised to wash the cabinet thoroughly to remove acid soldering fluxes before using.) This water and the walls of the cabinet are heated by means of the hot vapors rising from *Q*, at such a rate as to produce slight refluxing in condenser *B*. Because of the good insulation around the cabinet the "low heat" of a small hot plate, *T*, was found to be sufficient.

The humidity in the cabinet is maintained by admitting an excess of water into sedimentation bottle *A*. This bottle allows fine particles of dirt and rust to precipitate, so as not to plug the fine orifice at the bottom of *D*. The water on leaving *A* is used for cooling condenser *B*, then passes into the water pressure regulator, *D*. The excess water is allowed to escape by way of tube *F* to the drain. Water is then automatically admitted at a slow constant rate into mixing tube *K*.

An excess of air is admitted into air-pressure regulator tube *I*. The flowmeter, *G*, is calibrated to allow air, equivalent to 4 times the volume of the humidity cabinet, to pass through tube *E* per hour. The excess air is allowed to escape at the top of tube *I*. This air-pressure regulator tube will keep the rate of air flow into the cabinet constant regardless of relatively wide variations in laboratory or plant air pressures.

The metered air and water in tube *K* descend and enter the bottom of the cabinet at *P*, then travel a long upward spiral path through the submerged copper tubing. During this passage the air is preheated and prehumidified to the same conditions as the humidity cabinet before it emerges at the orifice in the center bottom of the cabinet at *N*. The air then rises vertically through the water in the bottom of the cabinet and finally emerges at the surface beneath the baffle plate, *M*. This baffle plate breaks all air bubbles and in so doing prevents the water spray from the breaking air bubbles from falling onto the test panels. It also serves to distribute the emerging air in a uniform manner throughout the cabinet and thereby prevent air channeling.

The exhaust air is allowed to escape through chimney *J*, which also serves as the handle for removing the lid. The underside of the lid is conical, so as to allow small amounts of water condensing on the lid to return to the bottom of the cabinet by way of the center. In this way the test panels will not be wet by water drops.

When the panel supports are removed for inspecting the test panels, the lid should be replaced as promptly as possible, if a very volatile heating medium is used. Any appreciable drop of temperature inside the cabinet will lead to air entering the jacket, and to consequent increase in evaporation losses. These, however, are very moderate. Even with the very low boiling cyclopentane (120° F.) the normal loss is less than 1 pint weekly.

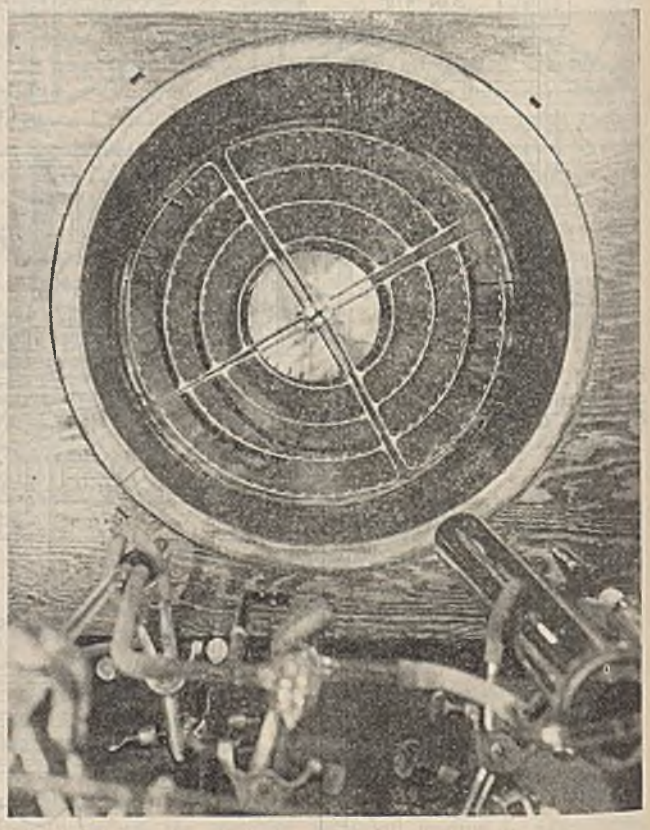


Figure 3

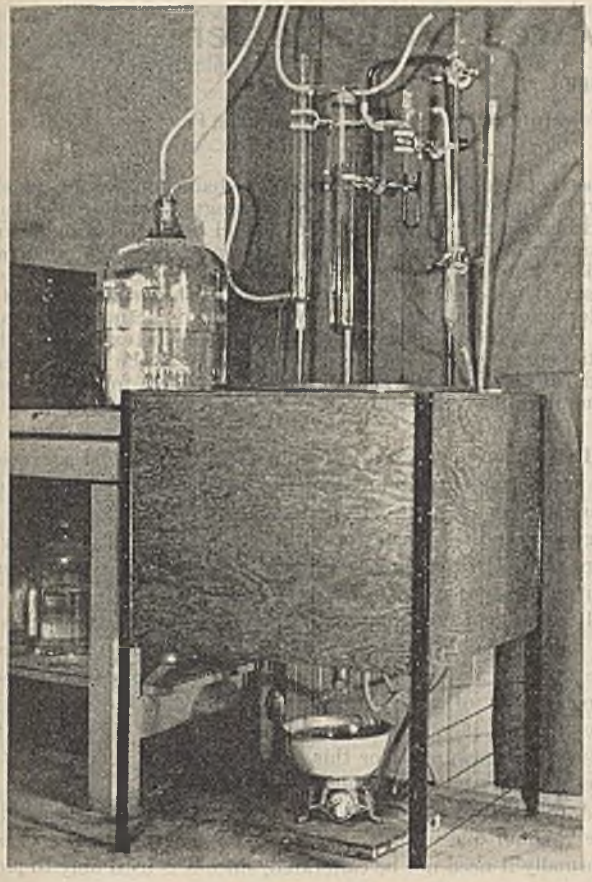


Figure 4

Test panels were suspended in different parts of this cabinet to check its uniformity; regardless of where they were suspended the corrosion was the same. Furthermore, because of the uniformity of the corrosion effects it was found that 1 × 3 inch test panels could be used with results fully as reliable as those obtainable with the usual 2 × 4 inch test panels. These smaller test panels permit a considerably larger testing capacity.

Table I illustrates differences in performance between the present cabinet and a cabinet of the conventional type.

Both cabinets were operated at 120° F. and 100% relative humidity. In the conventional cabinet moisture frequently condensed on the panels, and dripped down from them. In the cabinet here described some condensation took place when the cold panels were introduced into the cabinet. This initially condensed water remained as a dew on the panels, since evaporation could not take place at 100% relative humidity, but there was no continual condensation, nor any flow of water over the surfaces of the test panels.

The panels used in the test were prepared as follows: Test panels of 1 × 3 inches of S.A.E. 1025 sheet steel B. & S. gage 24 were cleaned by washing in benzene and wiping dry with a clean cloth. Completely new and uniform surfaces were exposed on the test panels by carefully buffing on a cloth buffing wheel (6 inches in diameter × 2 inches thick) coated with an abrasive, such as No. 300 grit Carborundum composition, similar to "Brushing Nu-Glu", as supplied by the J. J. Siefen Co., Detroit, Mich. Care was taken during this buffing operation to remove all sharp burrs at the edges of the test panels. The test panels were again wiped with a clean cloth to remove any traces of polishing grit and were then used. Care was taken not to touch the prepared test panels with the fingers.

All determinations were made in triplicate, and the figures shown are the average values. The variations of the triplicate determinations were within a range of 10% for the new cabinet and 28% for the conventional cabinet.

The fundamental difference between the cabinets is most clearly apparent from the two last items in the tabulation. If rust preventive "Q1" in its experimental stage had been evaluated on the basis of the conventional cabinet alone, it would have been discarded as no more effective than the sodium sulfonate or a fatty acid solution, and only half as effective as the older rust preventive "Q2".

Thus a product highly meritorious in its field of application would have been discarded.

However, the test in the new cabinet showed it to be at least 7 to 10 times more efficient than the petroleum sulfonate or the fatty acid, and at least 3 times more efficient than Q2, under conditions resembling ordinary factory storage, where a frequent flow of water over the surfaces of objects stored is out of question.

Corrosion resistance is a complex phenomenon, and a corrosion preventive which is better under certain test conditions is inferior under other test conditions, and vice versa. The same is true of actual storage under practical conditions.

A corrosion preventive which is most potent in actual use where continuous condensation of humidity takes place may be far from the optimum for storage in even extreme humidities without actual condensation; and a corrosion preventive which is best for the corrosive but not extremely humid atmosphere in steel plants is not best for storage under tropical conditions which are characterized by high temperature and humidity but where corrosive fumes are absent. A detailed analysis of the factors entailed would take us beyond the frame of the present subject; suffice it to say that any accelerated corrosion test must be adapted to the natural conditions in view. The cabinet here described will give an accurate reproducible measure of storage resistance under indoor conditions, in the absence of continuous precipitation or of corrosive vapors. The influence of these latter factors should be measured separately where they play a part, and should not be introduced where they do not enter into the applications envisaged.

Table I. Cabinet Performance

Material Tested	Time Required for First 3 Rust Spots to Appear on Surface of Iron Panels: Conventional cabinet	New cabinet
	Hours	*Hours*
Blank	10 min.	25 min.
Paraffinic mineral oil S.A.E. 40	4	7
10% butyl ricinoleate in Stoddard solvent	24	60
10% methyl oleate in Stoddard solvent	24	80
10% wood rosin in Stoddard solvent	28	65
20% sodium (petroleum) sulfonate in Stoddard solvent	120	236
10% oleic acid in 40 viscosity mineral oil	144	328
20% commercial rust preventive Q1 in Stoddard solvent	120	No corrosion in 2624 hours
20% commercial rust preventive Q2 in Stoddard solvent	240	840

ACKNOWLEDGMENT

The author takes this opportunity to express his sincere appreciation for the interest and cooperation which Johan Bjorksten, chemical director, Quaker Chemical Products Corporation, has given in the preparation of this paper.

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Apparatus for Surface Area Measurement

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Constructional details and method of use of a compact, rapid apparatus for the measurement of surface areas by low-temperature van der Waals adsorption are described.

SINCE the work of Brunauer, Emmett, and Teller (*1*) has established the use of van der Waals adsorption as a reliable method for surface area measurements, it is perhaps worth while to describe a simple and convenient apparatus which has been designed especially for such measurements.

APPARATUS AND PROCEDURE

The apparatus, as shown in Figure 1, consists of four parts: the adsorbent vessel, *A;* the gas buret with four bulbs, B_1, B_2, B_3, B_4; the mercury leveling flask, *D;* and the manometer arm, *E*. Its operation is as follows: With adsorbent in *A* and mercury level at *a*, the adsorbent is degassed through B_1, B_2 ..., and *E*. Then stopcock 1 is closed and the adsorbate—nitrogen, for example—is admitted by the same route. When the mercury level is raised above *a* and *E* is evacuated, the system acts as combined gas buret and manometer and the amount of adsorbate admitted can be calculated from the gas laws. Adsorption occurs when *A* is cooled in a suitable bath—liquid nitrogen, for example—to mark m_1 and stopcock 1 opened. By raising or lowering the mercury level to the five engraved marks, *m*, between the bulbs of the gas buret, five points on the adsorption isotherm can be determined.

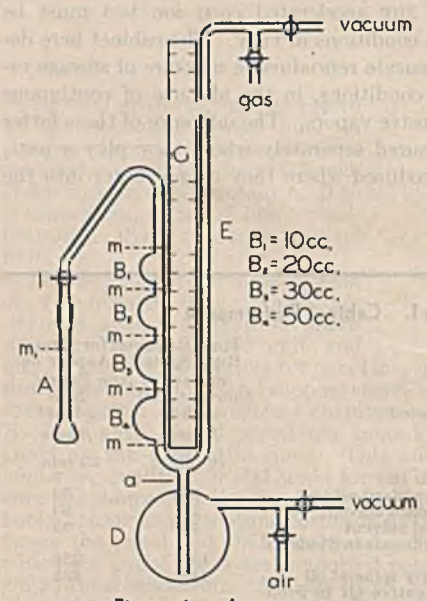

Figure 1. Apparatus

The volume from stopcock 1 to *A* must be determined by blank runs with *A* both at normal temperature and immersed in the refrigerant.

Figure 2 is a plot of the equation of Brunauer, Emmett, and Teller (*1*) for five bauxite samples prepared by various methods. Areas of the samples can be calculated from X_m by use of the data given by Emmett and Brunauer (*2*) and Livingston (*5*).

CONSTRUCTIONAL DETAILS

The adsorbent vessel should be conical, so that the adsorbent can be spread in a thin layer on the bottom, since deep layers of active adsorbents must be degassed very cautiously to avoid their being blown out of the vessel by the evolved gases. The use of a ground joint to connect *A* to the measuring system is very convenient, especially if a series of samples of known or constant density is being studied, for then only one "dead-space" determination need be made for the series.

The bulbs of the gas buret are flattened on one side to bring the engraved marks, *m*, between them close to the scale. This is especially important when the apparatus is intended for precision work, since it is then advisable to thermostat the apparatus and read the scale with a telescope. These bulbs should be constructed of heavy glass to avoid changes of volume due to varying internal pressure. The tubing connecting the bulbs and bearing the engraved marks should be of the same internal diameter as that used for manometer arm *E*—i.e., 7 to 8 mm. inside. Since the equation used for calculation of X_m is a straight line, only two points on the isotherm are required to determine it and therefore only one bulb is actually necessary. At least two are advisable, however, since the extra point thus obtained provides a check on the accuracy of the measurements and calculations. In the author's experience, four bulbs have sometimes proved useful.

The dimensions shown in Figure 1 are those found convenient for measuring surface areas of the order of 100 square meters, using nitrogen as the adsorbate and liquid nitrogen as the refrigerant, and the weight of adsorbent taken is adjusted to give approximately that area. Under these conditions a reproducibility of the order of 1 or 2% is to be expected.

If a different adsorbate or refrigerant is used, attention must be paid to the distance between the mark at the top of B_1 and the top of scale *C*. This distance must not be less than about three tenths of the saturation pressure for the combination chosen, and if possible should be somewhat more than the saturation pressure. For this reason the bulbs should be set as close together as practicable.

A McLeod or other low-pressure gage should be provided in the vacuum line in order to check completeness of evacuation. Normally it need not be calibrated, since it is used only to prove the attainment of a good vacuum.

MODIFICATIONS

Obviously this apparatus can be used for measuring ordinary adsorption where the pressures are in the centimeter range and an accuracy in pressure measurement not exceeding about 0.1 mm. is required. It is easily adapted for use with gases soluble

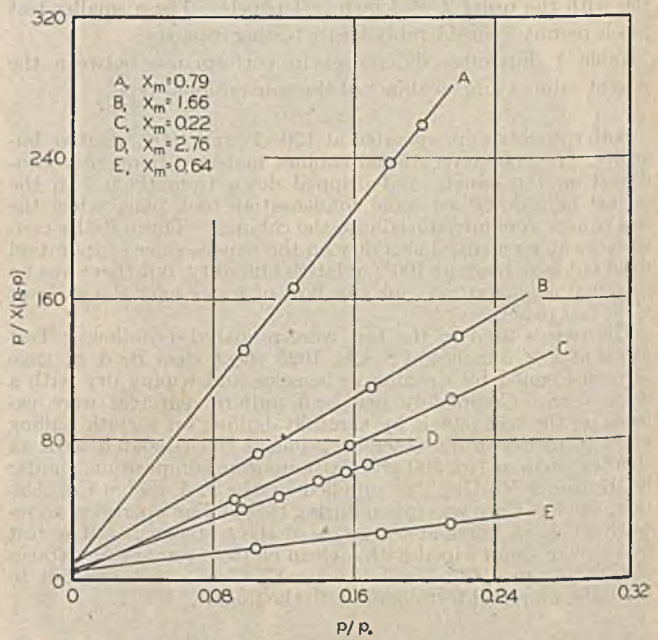

Figure 2. Plot of Equation of Brunauer, Emmett, and Teller for Five Bauxite Samples

X_m, Millimoles of nitrogen per gram of adsorbent
For curves *C* and *E* read 10 times the stated ordinate

in or reactive with stopcock lubricants, since stopcock 1 alone would have to be replaced by another closure (*3*). The use of a Pyrtz cut-off, for example, would eliminate both stopcock 1 and the ground-glass joint below it.

ACKNOWLEDGMENT

The author wishes to thank the Attapulgus Clay Company for permission to publish the data (*4*) of Figure 2.

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Improved Distilling Flask

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THE tendency of many substances to solidify on condensation, thus blocking narrow and inaccessible parts of a distilling apparatus, often leads to interruption of the distillation. To overcome this difficulty Anschütz (*1*) designed an all-glass distilling unit in which the receiver, acting also as condenser, was sealed on to the flask. This construction allowed the removal of obstructing material by heat applied externally. The scimitar-shaped receiver, however, which gives the name of sword flask to this type of distilling flask, was unsuitable for the collection of larger amounts of distillate. There was also a risk of contamination by accidental contact with the rubber stopper at the end of the receiver, and complications were encountered when in vacuum distillations the simultaneous use of thermometer and capillary was required.

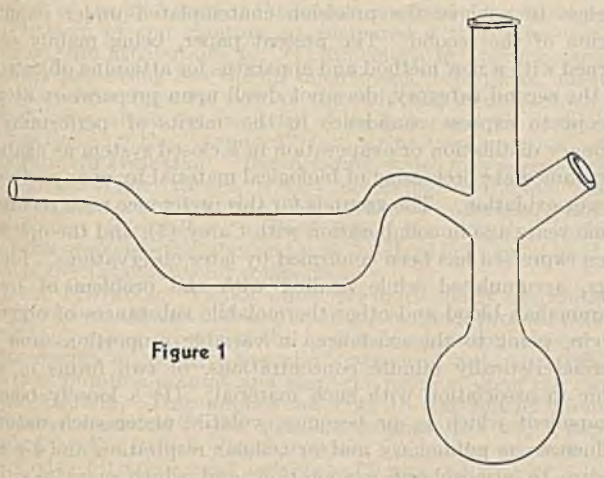

Figure 1

A few simple changes in the form of the Anschütz flask led to an improved distilling flask (Figure 1) of general applicability which has rendered valuable service over a number of years in distilling substances of low or high boiling point at either normal or reduced pressure. Two of the inconveniences encountered when using the Anschütz flask were overcome by giving the receiver a straight cylindrical shape of greater diameter and an outlet tube. These alterations, without unduly enlarging the receiver, considerably increase its capacity and prevent accidental contamination of the distillate by rubber. To accommodate separately a thermometer and a capillary a short side arm opposite the receiver and at the same height has been added. The thermometer and the capillary tube are held in place by rubber stoppers. If rubber must be avoided, the distilling flask shown in Figure 2 can be used with advantage, as it is especially designed for the distillation of corrosive substances. Both flasks, sturdy by virtue of design, are speedily assembled or dismantled and easily cleaned.

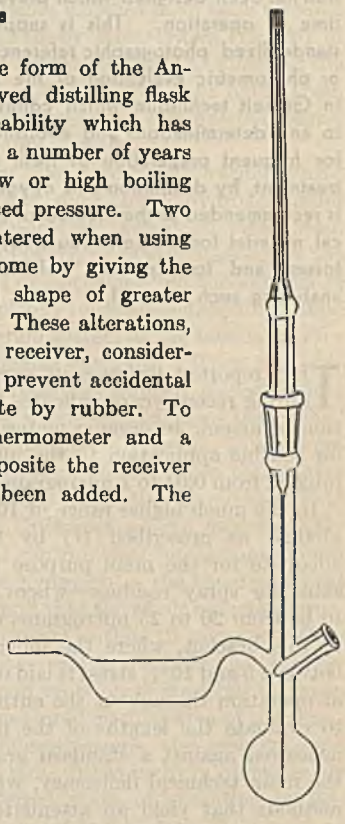

Figure 2

The approximate measurements for the different sizes of the distilling flask (Figure 1) commonly used in this laboratory are listed in Table I.

Ace Glass, Inc., Vineland, N. J., will stock this improved sword flask after the war, and at present is willing to fill any specific order for this item.

Table I. Dimensions of Flask

Capacity of Bulb	Capacity of Receiver	Neck		Distance of Delivery Tube and Side Arm from Bulb	Receiver		Side Arm		Outside Diameter of Distal End of Receiver
		Outside Diameter	Length		Outside Diameter	Length	Outside Diameter	Length	
Cc.	*Cc.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
4	4	1.2	6.5	1.5	1.6	3.0[a]	1.2	2.2	0.8
						4.8[b]			
10	10	1.6	8.5	2.0	1.8	3.5	1.5	2.5	0.8
						5.0			
50	30	1.6	11.0	3.5	2.5	6.0	1.5	3.0	0.8
						8.0			
100	60	1.6	12.0	4.5	3.2	8.0	1.5	3.5	0.9
						11.0			
300	160	2.2	13.5	5.5	4.4	12.0	1.7	5.0	1.0
						16.0			
550	300	2.5	15.3	5.5	5.0	15.5	2.1	5.0	1.0
						20.0			

[a] Measured along lower edge of receiver.
[b] Measured along upper edge of receiver.

ACKNOWLEDGMENT

The author wishes to express his thanks to Miss Marjorie Muir, Royal Ontario Museum, Toronto, for preparing the drawings.

The sealed-in capillary and thermometer jacket were added by J. C. Sowden, Toronto.

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Ultramicrodetermination of Arsenic by Gutzeit Spot-Filtration under Vacuum

A Rapid Technique Employing Photometric Calibration and Permanent Photographic Standards

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Deficiencies existing in various forms of Gutzeit procedure are examined in relation to the problem of developing a type of end reaction suited to quantitative determination of arsenic in the minute order of 0.04 to 1 microgram. To cover this range, a vacuum-accelerated Gutzeit reduction system for mercuric bromide spot filtration has been designed which prevents sources of error and shortens time of operation. This is supplemented with photoelectrically standardized photographic reference scales, adapted to either visual or photometric evaluation of the spot reactions, an improvement in Gutzeit technique which contributes precision and uniformity to end determinations and abolishes the time and labor required for frequent preparation of fresh standards. Immediate fractional treatment, by distillation and oxygen-bomb combustion of residues, is recommended as the method of choice in preparing fresh biological material for Gutzeit reduction, to prevent possible preliminary losses, and to separate "volatile" from "fixed" arsenic when analyzing such material.

THE reported progress in agricultural and food chemistry during recent years indicates that no method for determination of arsenic in organic material has yet proved satisfactory for routine application to the ultramicro range—for quantities ranging from 0.04 to 1 microgram of the elementary substance.

In the much higher range of 10 to 30 micrograms, the official method as prescribed (*1*) by the A.O.A.C. is undoubtedly adequate for the main purpose intended—the testing of fruit skins for spray residues—where the optimum range is stated to be from 20 to 25 micrograms of arsenic trioxide. But, even in this bracket, where the inherent error is estimated (*6*) as between 5 and 10%, stress is laid on the need for strict uniformity of operation throughout the entire procedure in order properly to evaluate the lengths of the paper-strip reactions as finally measured against a standard graph. This precaution denotes the main technical deficiency, which is common to all Gutzeit methods that yield an attenuated pattern of end-reaction as produced by flow of the reagent gas over narrow and elongated surfaces of the sensitized medium.

The task of extending application of the Gutzeit reaction to a more minute working order, while pursuing a "strip" or "string" technique, has embraced many perplexing difficulties, due to uncertainties in obtaining uniform sensitization of the paper, as noted by Cassil (*6*), and to other variable factors as discussed by Wichmann (*25–27*), and as instanced in a study of this problem by How (*13*) and reviewed by Wichmann (*26*). The prospect has consequently been suggested that less delicate end reactions for arsenic, such as the cerulean-molybdate colorimetric methods of Klein and Vorhes (*16*) or of Hubbard (*14*), or even the iodine titrimetric methods (*7*, *8*), might more easily be adapted to ultramicro extension than the Gutzeit method.

Investigation of fresh and unstable organic material in the field of clinical pathology, immunology, and physiological chemistry has demanded the use of an ultramicromethod for quantitative determination of arsenic in the very minute order of 0.04 to 1 microgram. This limitation is due to the small size of test samples of biological material which it is necessary or desirable to examine in this field: blood, glandular secretions, cerebrospinal fluid, 1 to 2 ml.; urine, liquid culture media, etc., 5 to 10 ml.; expired air, 5 to 10 liters; animal danders, bacterial allergens, etc., 15 to 25 mg.; vegetable pollens, 25 to 50 mg.; house dusts and tobacco, 25 to 200 mg.; fresh biopsy and necropsy tissues, 100 to 500 mg.

Accuracy in analyzing such small samples of organic material for contained arsenic requires:

1. Avoidance of errors due to unsuspected changes of arsenic content during preparatory processes, whether such processes involve the laborious procedures of wet-oxidation or the more rapid procedure of dehydration followed by flame combustion in oxygen.
2. A method of isolation and final determination which will minimize reagent impurities and be capable of showing clearly the presence of 0.01 microgram of elementary arsenic, while regularly giving blank determinations below 0.04 microgram, and provided with a system of evaluation having a margin of error not exceeding ±3%, or visually perceptible differential values of 0.05 microgram in the optimum range up to 0.50 microgram, and with opportunity for further refinement by photoelectric photometry.

Unless error be avoided under the first category, it will be useless to achieve the precision contemplated under requirements of the second. The present paper, being mainly concerned with a new method and apparatus for attaining objectives of the second category, does not dwell upon preparatory steps, except to express confidence in the merits of performing a primary distillation or evaporation in a closed system as against the immediate processing of biological material by open methods of wet-oxidation. The grounds for this preference were reported some years ago in collaboration with Carey (*4*), and the opinion then expressed has been confirmed by later observations. Findings, accumulated while dealing with the problem of fresh mammalian blood and other thermolabile substances of organic origin, point to the existence, in variable proportions and in characteristically minute concentrations, of two forms of arsenic in association with such material: (1) a loosely bound component which is, or becomes, volatile under such natural influences as pulmonary and/or cellular respiration and by exposure to atmospheric evaporation, and which may be artificially separated from such material by drying or distillation at 56° C. in a current of molecular oxygen; and (2) a relatively fixed or thermostable component which is not removed from the material by heating at 56° to 80° C. under the same conditions.

Since these observations derive largely from investigations of clinical material, they will be reported in detail elsewhere and are here briefly summarized only to serve as a premise for pointing out that the ultramicrodetermination of arsenic which is found in association with biological substances may be of physiological interest and not necessarily confined to toxicology.

The authors' interest has been mainly concentrated on arsenic transformations in organic material under natural conditions, as through oxidation-reduction phenomena in physiological systems or through simple exposure to atmospheric oxidation, and not on the artificial effects of destructive analysis by means of powerful oxidizing agents.

The mechanism of dissociation of minute amounts of arsenic by evaporation at 56° to 60° C. and its recovery from fresh biological material by acid extraction of the vapors is not clear, since the composition of the volatile product has not yet been

determined. It is presumed to be derived by the splitting off of a labile arsenic group from an arsenoprotein, but whether this is an arsonium group, an alkyl arsine, or simply arsenic trihydride is not known. The arsenic content of the volatile fraction in proportion to that of the dried residue of human blood, is very variable, and seems to depend on age and activity of the organism, or more specifically on body metabolism. For example; in 212 analyses of human blood taken from 51 adult individuals, the average "total arsenic" found was 59.14 micrograms per 100 cc., ranging from 10 to 190 micrograms, with the proportion of "volatile arsenic", as defined above, averaging 40.57% and varying from 0 to 100%. These variations were, of course, greater for the entire series than for any individual of the group, which was composed of 33 ambulant patients, suspected or definitely diagnosed as "chronic arsenical poisoning" or "arsenic retention", 13 cases "for diagnosis", 2 cases of "hyperallergic state", and only 3 "normal" healthy individuals. Of these latter, 2 had an average "total arsenic" in their blood of 25.5 micrograms per 100 cc. with 85% "volatile", and the third normal individual (in the 28th week of pregnancy) showed a total blood-arsenic of 180 micrograms per 100 cc., which corresponded to the physiological increase during gestation, as shown by Guthmann and Grass (*12*). In this last case the interesting features were that the "volatile arsenic" fraction was 100%, and the expired-air test, performed at the same time, showed 50 micrograms of arsenic per 100 liters as collected directly through the vacuum extraction train, and was the highest test on exhaled air which has been recorded in the authors' series.

There can be no doubt that the sensitiveness of the Gutzeit arsine–mercuric bromide end reaction is, per se, sufficient to meet the exacting requirements indicated above. There are, however, certain faults in the accepted Gutzeit techniques as affecting ultramicrodeterminations which demand correction in order to extend its usefulness to that range.

Paper-Strip Method. After several years' experience with Gutzeit reactions produced by the paper-strip method it became evident to the authors that the inaccuracies and disadvantages of this system of end reaction, as compared with the paper-disk method, are attributable to: (1) difficulties in appraisement of stains representing less than 2 micrograms, because of unequal distribution on opposite sides of the strip and inconspicuous intensification along the free edge of the paper and (2) instability of reactions on the "standard" test strips used as criteria for quantitative evaluation. Rapid fading, which may escape attention in the higher ranges, is very noticeable in the low values and renders such strips unreliable as scales of reference within a few hours. Here follows a disadvantage common to both strip and disk systems—the labor and time involved in frequent production of fresh standards for comparison.

Disk Methods. Faults which are particularly attributable to the various Gutzeit disk or diaphragm methods proceed mainly from the heating process at the hydrogen generator, a necessary feature with all positive-pressure systems of disk filtration in order to obtain a prompt and clear-cut reaction upon the sensitized medium. This applies not only to the smaller (5-mm. diameter) areas and to the standard 6.5-mm. disk which is prescribed by the British Pharmacopoeia (*3*) but to methods (*17*) employing filtration areas as large as 20 mm. in diameter. Boiling or lesser heating of the zinc-acid mixture is undesirable since it not only causes irregular action with fluctuating pressures within the confines of a small generating vessel having a resistant outlet, but produces an uneven flow of gas and a tendency to excessive heat at the absorption level. This has in some instances (*2*) been lessened by use of a manometer side arm attached to the generator which provides some cushioning effect on pressure oscillations. In other forms of apparatus (*17*) irregularities are lessened while the total pressure within the system is raised, by forcing hydrogen or nitrogen gas into the generator from outside the system.

Some analysts (*11*) place a heating limitation of from 40° to 60° C. in the reducing vessel and aim to promote a rapid evolution of hydrogen gas by means of catalytic "impurities" in the zinc reagent, either accidental ingredients, or purposely alloyed with the zinc, as for example, 0.3% of copper (*24*). Other analysts (*10*, *20*) insist on a relatively slow rate of gas evolution and rely mainly on a stannous chloride activation of the zinc for augmenting activity of the hydrogen gas so produced.

Gutzeit disk systems under varying degrees of positive pressure, as commonly practiced, thus appear to present a dilemma, in that they require heat to accelerate gas formation and to maintain sufficient pressure to force a current of gas through the filter medium against the internal resistance of the system plus atmospheric pressure beyond the filter disk; while, on the other hand, this same internal pressure factor works against the evolution of gas at the source and raises the boiling point in the generator. Irregular gas production thus becomes inevitable and results in an intermittent or irregular delivery of gas at the reaction level of the filter disk, while excessive heat in the generator carries water vapor to all levels. These conditions tend to wash away some of the water-soluble arsenic-mercury halide stain after its deposition on the filter-disk, causing a marginate diffusion of the reaction or uneven staining. Such effects are lessened, however, by cooling devices placed at or just below the absorption level.

Finally, there exists in all Gutzeit systems which operate under positive pressure the possibility of leaky joints permitting small losses of arsenic trihydride to the atmosphere. Alleged (*15*) partial losses of arsenic hydride at the absorption level by passage through the sensitized filter disk, with failure to react on the mercuric halide, probably do not occur in ranges of concentration below 10 micrograms, even under boiling conditions, as was shown long ago by Bird (*2*) and later by Cribb (*10*).

While most of these objectionable features have been stressed by early workers (*23*), recognized by later critics (*19*, *20*) of Gutzeit technique, and to some extent corrected as affecting the semimicro range of determination, the authors have found them seriously detrimental in the micro range and a complete obstacle to successful practice in the ultramicro range.

After experimenting for more than a year with various forms of electrolytic cells in seeking to produce atomic hydrogen under instrumental control, the complicating factors so introduced, especially the unpredictable influence of overvoltage upon the more sensitive (mercury) types of electrodes, led the authors to abandon hope of finding a completely reliable electrolytic method and to concentrate upon a vacuum system as the best means for correcting the faults of the zinc-acid generator. They then discovered that a system of spot-filtration securely closed against leakage could be ensured under a low vacuum, and that, when operated at from 0.25 to 0.5 atmospheric pressure, this system would yield more uniform and prompt end reactions with better control of heat and moisture at the absorption level than could otherwise be produced upon a Gutzeit-sensitized medium. By this means, under instrumental control, they furthermore found that the development of active hydrogen in the usual zinc-acid mixture is markedly increased and that the whole reduction process proceeds evenly to a conclusion within 15 minutes. Gas-current impedance in the scrubber, and at the filter disk, is overcome by this device and the end reaction is rendered in sharp demarcation upon a precisely measured circular area of the sensitized paper. For ultramicrodeterminations, the reactive area may be reduced as low as 3.17 mm. (0.125 inch) in diameter, or it may be suited to any preferred range of operation by adjusting the caliber of the filtration jet to 6.35 mm. (0.25 inch) or larger, with corresponding adjustment of the vacuum.

Realization of these characteristics finally provided an opportunity for applying photographic and photometric methods to quantitative evaluation of the Gutzeit end reaction, a project found impracticable with either strip or thread reactions. The use of photographic step scales for permanent standards of reference, as here reported, has now been practiced in this laboratory for more than two years; it is so far beyond the experimental stage that the authors do not hesitate to recommend it as an important time- and labor-saving device which also implements precision in the procedure here described. These artificial standards of reference consist of an accurately graded series of silver deposits photographically printed on bromide paper from a master negative. The fineness of gradation may be varied to suit special requirements (or limited ranges), as is shown in Figure 4.

REAGENTS

Sulfuric Acid, c.p., special arsenic-free (supplied by the J. T. Baker Chemical Co.), obtained in 500 ml. Pyrex bottles, usually rated on label as having less than "0.000000% As".

Tolerance: 10 cc. in 90 cc. of double-distilled water with 5 cc. of potassium iodide solution, 7 grams of zinc and 0.5 cc. of stannous chloride solution in reduction system under vacuum of 0.33 atmosphere (250 mm. of mercury) for 30 minutes and with intervention of lead acetate solution in absorption tube for removal of hydrogen sulfide, should show less than 0.02 microgram of arsenic, or 0.0000002% of arsenic in a 10-cc. sample of acid.

Hydrochloric Acid, c.p. special arsenic-free (J. T. Baker Chemical Co.), obtained in 500-ml Pyrex container as above, and usually rated as not more than "0.0000001% As". Tolerance: 10 cc. in 90 cc. of double-distilled water with 5 cc. of potassium iodide, 7 grams of zinc and 1 cc. of stannous chloride under vacuum in Gutzeit apparatus, as above, should yield a similar blank.

Zinc Metal, c.p. special, mossy, for microdetermination of arsenic (J. T. Baker Chemical Co.). The mossy variety of zinc is preferred because it seems to be more consistently free from arsenic than the "granular 20-mesh" variety, although both are claimed by the manufacturers to contain less than "0.000001% As". Tolerance: 7 grams, after preliminary treatment as below for 5 minutes to remove any possible surface contamination from atmospheric adsorption, should be submitted to same tolerance test as hydrochloric acid above and should show less than 0.02 microgram of arsenic. Approximately 7 grams should be used in each arsenic determination having 100 cc. of acid solution in the reduction flask. Such portions, before use for arsenic determination, should always be freshly cleansed, activated with 50 cc. of hydrochloric acid diluted 1 to 3 with double-distilled water and with admixture of 2 cc. of stannous chloride for 5 minutes under vacuum, and then washed in double-distilled hot water. Just before use in a determination the zinc should again be washed in double-distilled water.

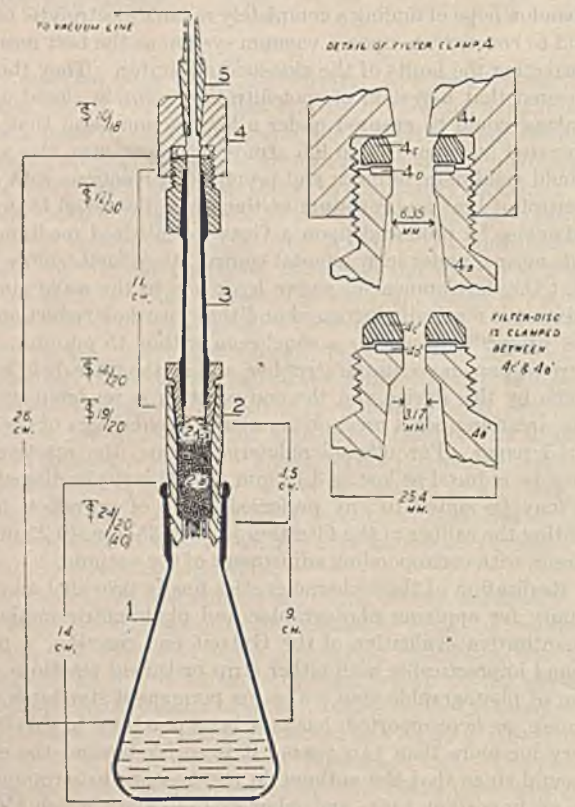

Figure 1. Diagram of Apparatus

1. Pyrex flask, 200-ml. capacity
2. Absorption tube, machined from methyl methacrylate resin
   2A. Pyrex wool
   2B. Pyrex, granular, 10- to 20-mesh, saturated with $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ solution
3. Pyrex delivery tube with ground-glass 14/20 and 12/30 joints
4. Filter clamp assembly (methyl methacrylate resin)
   4A. Upper member
   4B. Lower member with 6.35-mm. jet
   4B'. Lower member with 3.17-mm. jet
   4C, 4C'. Compression washers
   4D. Metal seat, central opening precision-reamed to 6.35 mm. and gold-plated
   4D'. Same as 4D, 3.17-mm. opening
5. Connecting tube for vacuum line (methyl methacrylate resin)

Double-Distilled Water. This should give blank test as above with the other reagents.

Potassium Iodide, c.p., 15 grams dissolved in 100 cc. of double-distilled water. This solution in 5 cc. amounts should give a blank test as above with the other reagents.

Stannous Chloride, dihydrate, arsenic-free. A 40% solution of this in hydrochloric acid is required. Tolerance: 1 cc. in 10 cc. of hydrochloric acid and other reagents and conditions as stated under hydrochloric acid should yield blank test in the same order.

Lead Acetate Solution, 10 grams of lead acetate trihydrate in 90 cc. of double-distilled water, made acid to litmus with acetic acid and then made up to 100-cc. volume. One to 3 cc. of this solution poured upon 15 grams of granular Pyrex in absorption tube (see apparatus), with reaction conditions as described under other reagents, will involve some seepage into the reduction flask. It should yield a blank reaction in the same order.

Pyrex, Granular, obtained from the manufacturer in fragments between 10- and 20-mesh. This should be sifted to size and then thoroughly cleaned with nitric acid, rinsed with sodium hydroxide solution, and washed with hot distilled water, then dried in an electric oven before use. Under conditions of repeated use it is best left in the apparatus, a 15-gram portion being placed in the hydrogen sulfide–absorption chamber (2, Figure 1) and cleaned after each run (or when blackened by deposit of sulfur) by saturating with 10% nitric acid, neutralizing, and washing.

Pyrex, Fiber, known commercially as No. 719. A small pledget, previously impregnated with lead acetate solution and allowed to dry, is placed in the absorption tube on top of the Pyrex granular preparation.

Mercuric Bromide, c.p. A 5% solution in 95% ethyl alcohol is used for sensitization of the filtration disks as described below.

Whatman No. 40 Ashless Filter Paper, made by Balston & Co., Ltd., black label, cut into disks with a 1.4-cm. ($^9/_{16}$-inch) punch and sensitized as directed below.

Ammonia Solution, 10%, made by adding 10 cc. of concentrated ammonium hydroxide (d. 0.880) to 20 cc. of 95% ethyl alcohol, in a dropping bottle.

Standard Volumetric Solution of Arsenic. This is made by dissolving 1.3204 grams of c.p. arsenic trioxide in 25 ml. of a 20% arsenic-free sodium hydroxide solution, saturating this with carbon dioxide, and diluting with double-distilled and recently boiled water to exactly 1000 cc. in a volumetric flask at the standard temperature. From this stock solution, which contains 1 mg. of elementary arsenic (atomic weight 74.91) per cc. at the standard temperature, further dilutions are made with the precaution of sodium sulfite reduction just before use, as recommended by How (*13*). Dilutions to a final titer of 0.1 microgram of arsenic per cc. are made for use in the ultramicrocalibration of the standard step scales; these dilutions must be conducted with the utmost precision.

Sensitization of Filter Disks. A supply of the filter disks, enough for a week or 10-day period, is placed in a wide-mouthed bottle with tubulated stopper, or in a micro filtering flask, and this is half-filled with the mercuric bromide solution. The disks are thoroughly impregnated with the solution and all air is removed from the pores of the paper by exhaustion under a vacuum of 75 to 100 mm. of mercury for 2 hours according to the method of Cassil (*5*). The mercuric bromide solution, unless recently made, should be filtered through a folded filter as practiced by Rosenfels (*22*) before being used; it should be discarded entirely when 2 months old. The sensitized disks, in an amber-colored bottle, holding the solution, may be safely kept for a week or 10 days if protected from bright light. Just before use they are removed from the bottle, dried in the air on filter paper, and used very promptly; at this stage they should again be protected from bright light and never returned to the bottle, but discarded if not used. Optimal sensitiveness and the proper degree of porosity depend on careful technique in all these details.

For testing the sensitized disks for undue porosity under vacuum-filtration or permeability to excessive amounts (2 to 4 micrograms) of arsenic in the test solution, the authors have superimposed a secondary or telltale disk above the primary disk, but never have observed any sign of arsenic trihydride leakage or telltale staining.

APPARATUS

The apparatus is diagrammatically shown in Figure 1. Its laboratory set up for operation in duplicate units is illustrated in Figure 2.

The hydrogen generator is a 200-ml. Erlenmeyer Pyrex flask with a 24/40 $ ground joint for connection with the hydrogen sul-

fide scrubber, 2. This part is taper-turned from methyl methacrylate resin, 2.5-cm. (1-inch) rod stock, with a 24/40 ⅀ lower extremity and relief groove to fit the flask (1, Figure 1), and its bottom is drilled symmetrically with 19 holes of 1.2-mm. diameter. Its cavity has an inside bore of 16 mm. in its lower (cylindrical) portion and is tapered 19/22 ⅀ in its upper portion to receive a stopper which is taper-turned out of the same material to 19/17 ⅀ on the outside. This stopper is drilled and taper-bored to 14/28 ⅀ to receive the tube and stopper assembly. This forms a chamber to contain a 15-gram portion of granular Pyrex, 10/20-mesh, 2*B*, which is saturated with lead acetate solution and surmounted by a pledget of Pyrex wool, 2*A*, impregnated with the same reagent, dried.

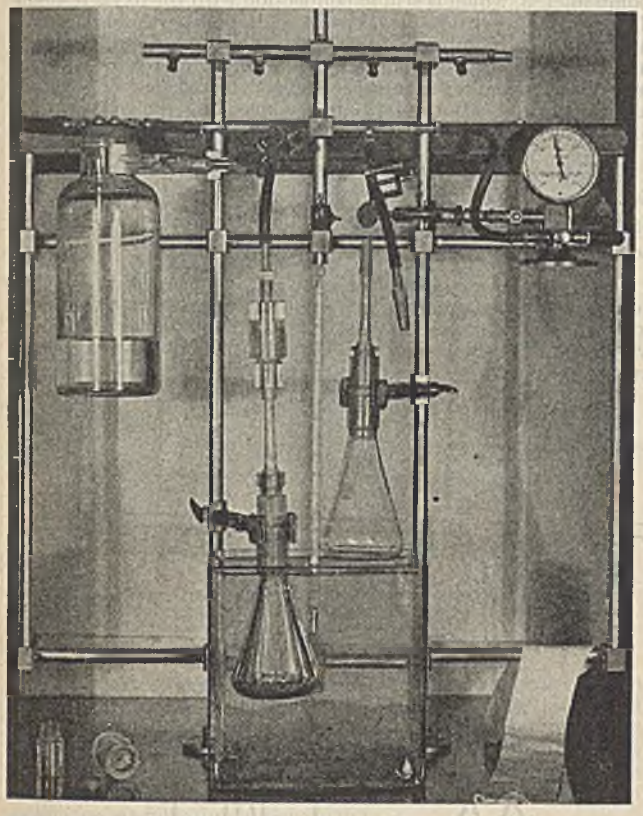

Figure 2. Apparatus

The Pyrex delivery tube, 3, has an upper 12/30 ⅀ ground joint and a lower 14/20 ⅀ ground joint to fit parts 4*B* and the stopper of 2, respectively. Its internal diameter is approximately 8.5 mm. at the lower end and 6.5 mm. at the upper end; its length is 11 cm.

The filter clamp assembly consists of 2 members: the lower clamp member 4*B*, machined from 2.5-cm. (1-inch) methyl methacrylate rod stock, threaded ¾-inch, 16 threads per inch, Amer. Std. machine screw thread, drilled centrally and taper-bored 12/30 ⅀ to fit upper joint of 3. Its upper surface is countersunk 0.1 cm. (0.040 inch) deep by 1.24-cm. (0.495-inch) diameter to receive a gold-plated silver washer 1.25-cm. (0.500-inch) diameter, 0.15 cm. (0.060 inch) thick. This washer (before gold-plating) is bored and precision-reamed centrally to 6.35 mm. (0.250 inch) and given a slightly tapered outer edge for tight push-fit into 4*B*, thus forming a permanent precision-gaged gas jet for 6.35-mm. spot-filtration. It also provides a nonadherent gastight seat for contact with the under surface of the mercuric bromide-impregnated filter disk, which is held in compression against it by 4*C* under clamping action of 4*A* (see detail, Figure 1). 4*B'* is an exactly similar lower clamp member provided with gas jet for 3.17-mm. (0.125-inch) diameter spot-filtration.

4*C* and 4*C'* are compression washers, precision-surfaced and machined from 1.9-cm. (0.75-inch) methyl methacrylate rod stock, 1.4-cm. (9/16-inch) outer diameter and drilled centrally 0.6 and 0.3 cm. (0.25 and 0.125 inch), respectively, to correspond with 4*B* and 4*B'*. These compression washers are tapered at the upper edges to present a circular bearing surface 0.3 cm. (0.125 inch) wide for screw compression by 4*A*.

The upper clamping member, 4, 4*A*, is machined from 3-cm. (1.25-inch) methyl methacrylate rod stock and has a ¾-inch × 16, Amer. Std. machine screw thread to fit 4*B* and 4*B'*. It serves for compressing washers 4*C* and 4*C'*, thus effecting a marginal seal for the sensitized filter disk when seated upon the gas jet of the lower clamping member. It has a precision-surfaced bearing for contact with compression washers 4*C* and 4*C'* and is bored centrally with a 0.6-cm. (0.25-inch) hole and then taper-bored 10/18 ⅀ to give outlet for gas through connection with 5.

The connecting tube to vacuum line, 5, is machined from 1.25-cm. (0.5-inch) methyl methacrylate rod stock by boring centrally with a 0.3-cm. (0.125-inch) hole and taper-turning on the outside 12/30 ⅀ at the proximal end for connection with 4 and distally for connection with rubber pressure-tubing of the vacuum line.

The vacuum line may be actuated by a laboratory motor pump, or an ordinary water pump used for filtering may be attached, provided that the water pressure is adequate and fairly constant. The latter form of pump will, at best, not operate more than two filtration units simultaneously. In any case there should be a water trap in the line, as shown in Figure 2, and a convenient form of vacuum gage should be used, provided with an air-intake valve for control of pressure reduction in the system. During hot weather it is advisable to use a cooling jacket of moistened cotton stockinet (an infant's size no. 4 stocking with toe removed answers well); this should surround the delivery tube and clamping mechanism and be evaporated in the air current of an electric fan.

METHOD

In planning a method for isolation and determination of arsenic as existing in organic substances in unknown combination, not only must the problem of cyclic compounds resistant to oxidation be met, but the problem of thermolability and unknown vapor pressures of component arsenic groups, which may volatilize under natural conditions, must be considered, especially in ultramicrodeterminations. In dealing with fresh animal tissues and with other kinds of unstable organic material, it may therefore be well to avoid the idea of "preparatory" treatment being a detached process, and to substitute the concept of a definite analytical partitioning of procedure, *ab initio*. The authors recommend two analytical steps in examining such material, both leading to a test solution suitable to Gutzeit reduction. The first is directed towards capturing any heat-sensitive arsenic component, in however minute concentration, by evaporation in a distilling system. The second step is to isolate the relatively "fixed" arsenic component which remains in the dehydrated residue, and which may be resistant to ordinary methods of oxidation, by submitting this residue to oxygen-bomb combustion. The two solutions which result from this partitioning may be separately tested for their arsenic content, or they may be combined for determination of "total arsenic" in the sample.

1. A measured sample of the material is taken in as fresh and natural condition as may be obtainable. For example, 2 cc. of circulating blood are withdrawn from the vein and are at once deposited through the needle of an aspirating syringe upon a small bundle of arsenic-free absorbent cotton suspended within a distilling flask (A_2, Figure 3) which is already connected with the vacuum-operated absorption train. On removing the needle from the side tubulature of flask A_2, this opening, 1, is screened with a cotton filtering plug (as shown at 1*A* in the larger flask, A_1) to prevent the possible access of any dust or extraneous matter from the oxygen line, and is at once connected with a controlled supply of pure dry oxygen gas as obtained from liquid air. A minimum flow of this gas is now started while the flask is heated on a water or sand bath to 60° C. The flow of warmed oxygen gas, aided by suction of a vacuum pump at the end of the train, removes all volatile products and moisture from the preparation under a reduction of pressure which can be varied throughout the operation to suit the optimum speed of evaporation. The vapors are conducted from the flask through the acid-nebulizing jet, 1*B*, which sprays hydrochloric acid (1 to 1) into the mixing chamber, 2; here they are chlorinated and mixed with a cloud of ammonium chloride which is produced by ammonia gas derived from the reservoir, 3, containing ammonium hydroxide, which reagent, by adjustment of the rotating stem, 4, may be varied as to its surface area. This adjustment is to provide a visible cloud of

ammonium chloride in the combined vapors to serve as an indicator of the rate of absorption throughout units *C*, *C'*, and *D* of the train, all of which contain sulfuric acid (1 to 3).

The temperature in the distilling flask is maintained at 60° to 80° C. until there remains only a desiccated residue. In the case of 2-cc. samples of blood, this procedure requires about 30 minutes and somewhat longer for similar amounts of other animal and vegetable tissues. For larger (5- to 15-cc.) samples of liquids, such as urine and liquid culture media, a larger flask, A_1, with reflux condenser is used, and evaporation is proportionately prolonged. The heat-labile components and most of the moisture having been evaporated and extracted in the absorption train, the vacuum line and oxygen supply are disconnected. All extracts, with washings from the train, are then made suitable to Gutzeit reduction (12% acid concentration) by dilution to a volume of about 100 cc. This constitutes a test solution representing the first or "volatile" fraction of the sample under analysis.

2. In the second analytical step, the dehydrated residue—consisting, in the case chosen, of dried blood on cotton—is promptly removed from flask A_2, Figure 3, and placed in the cradle fixture, 13, of the oxygen bomb, A_3, where its contained fuse wire, 4, is connected to the terminals, 10*A* and 11, of the ignition system in the bomb head, 1. The cylinder or combustion chamber, 2, supported by its clamping collar (not shown) is then raised to the closed position as indicated in A_3, and there securely sealed by a massive clamping mechanism (not shown). The bomb is then charged with oxygen at 17.6 kg. per sq. cm. (250 pounds per square inch) pressure and the charge ignited by closing the ignition circuit. This process is essentially as described in a former report (*4*), but with certain improvements in apparatus (manufactured by the Parr Instrument Co., Moline, Ill.) of more recent development.

After the combustion, which is practically instantaneous, the gases and smoke are completely exhausted from the bomb through its outlet, 4, which is connected to the same absorption train, *B*, *C*, *C'*, *D*, as described in the first instance. In this case the ammonium ions which are given off from the ammonium hydroxide in the communicating reservoir, 3, of the nebulizing chamber, *B*, 2, combining with the spray of hydrochloric acid, serve primarily to effect an ammonium chloride cloud precipitation of all smoke particles within the globular portion of this chamber. Thence the combustion gases are conducted under action of the vacuum pump into the remaining units of the extraction train which have been provided with the same reagents used in the previous operation. When the exhaustion is completed, any residual gases in the bomb are washed through the train by a current of oxygen admitted through the bomb inlet, 3, which is still in connection with the oxygen supply. The resulting extracts, combined with bomb residues and all washings from bomb and train, are similarly made up to 100 cc. in volume to form another test solution which represents the second analytical fraction, or relatively "fixed" arsenic, of the original sample. Both test solutions may be reduced separately, or combined and determined as "total arsenic".

Isolation and Determination of Arsenic from Test Solutions. Whatever the previous analytical procedure may have been, the test material is assumed at this stage to be in acid aqueous solution.

The degree of acidity, if not known, should be determined and adjusted to approximately 12% hydrochloric acid, when brought to 100 cc. in volume. This test solution, containing the arsenic which is to be isolated as arsine and then quantitatively determined, is placed in the hydrogen generator flask (1, Figure 1)

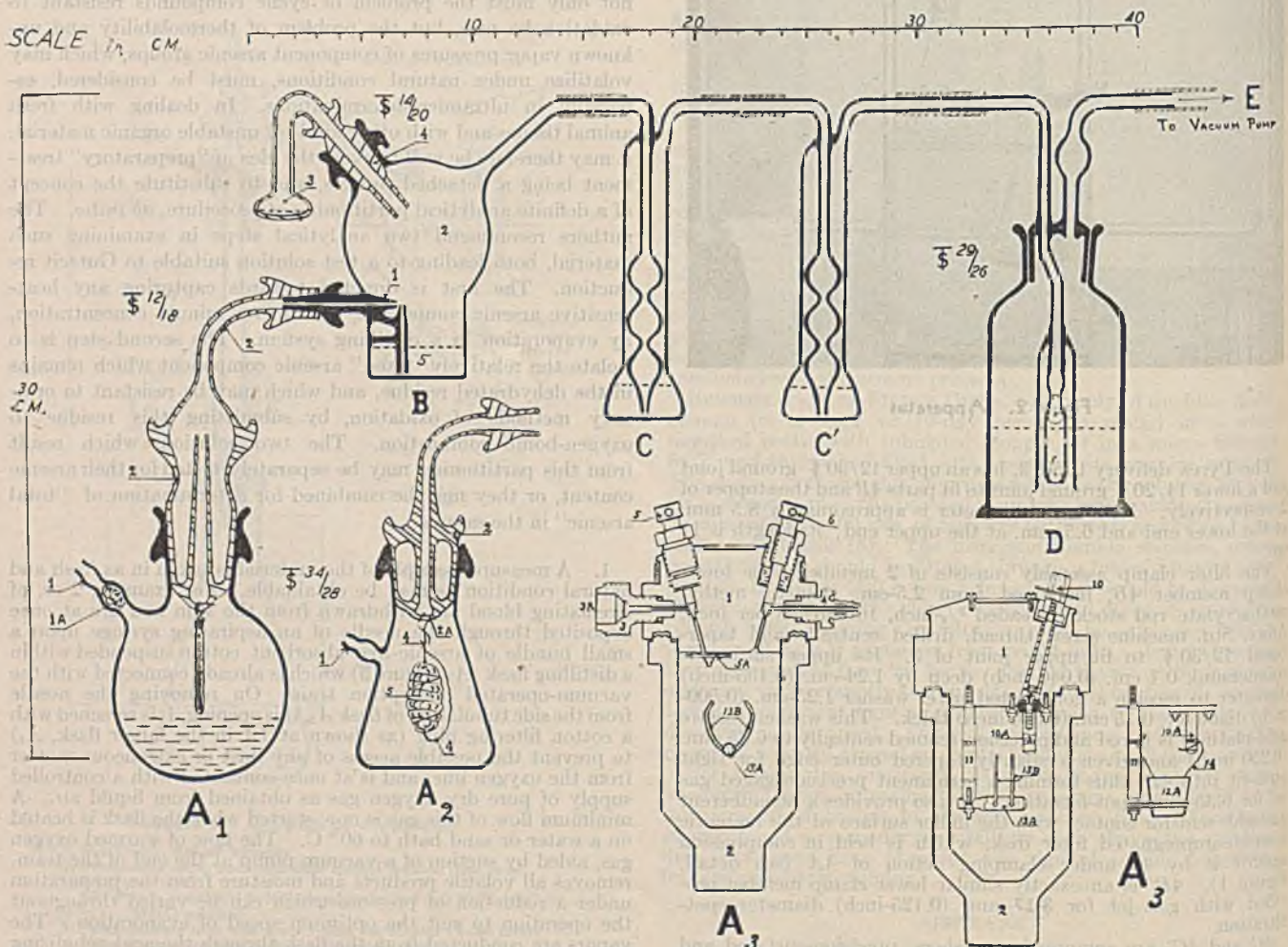

Figure 3. Apparatus for Separate Determination of Volatile and Fixed Arsenic in Small Quantities of Biological Material

A_1, A_2. Flasks for distilling and dehydrating material for analysis. A_1 is for 5- to 25-cc. samples of urine, liquid culture media, etc. A_2 is for smaller samples, 2 to 2.5 cc., or grams, of blood, fresh tissues, etc.

A_3, A_3. Right sectional views of oxygen bomb used for flame combustion of dried residues

B, *C*, *C'*, *D*, *E*. Vacuum extraction train used in connection with A_1, A_3, and A_3 for obtaining test solutions for ultramicrodetermination of arsenic by extraction of volatile products of evaporation or by extraction of smoke and gases from oxygen-bomb combustion of dry residue

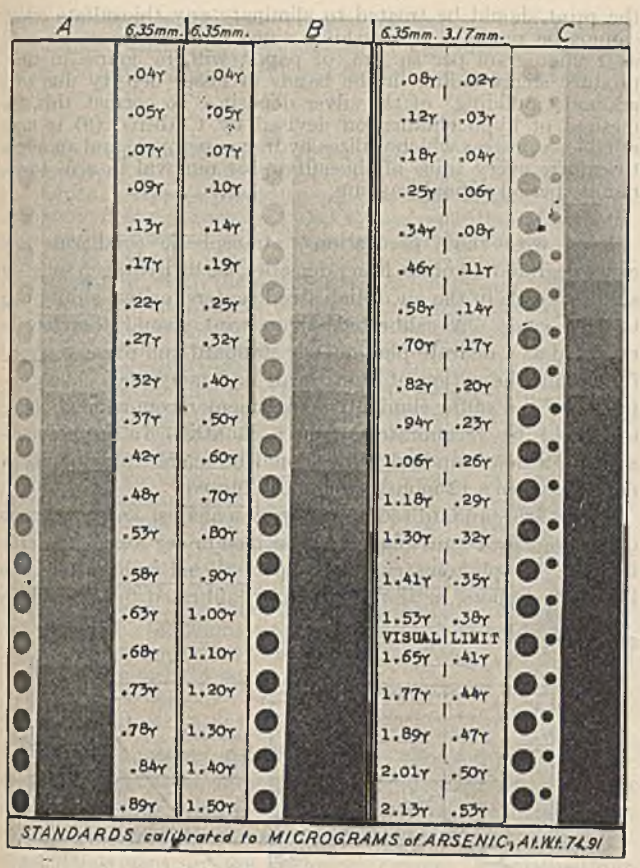

Figure 4. Photographic Standards for Vacuum-Gutzeit Spot Reactions

Critical reproduction of original is limited by photomechanical processes involved. The original step scales present 3 series of sepia tints; each series has a less steep gradient of intensity than is represented in the illustration.

and 5 cc. of potassium iodide with 1 cc. of stannous chloride are added. The flask is then placed in a water bath at 15° C. and 7 grams of activated zinc are added; it is then very promptly (10 to 15 seconds) connected to the other parts of the apparatus which have previously been assembled and supplied with reagents, including a sensitized filter disk securely clamped in position for gas filtration under vacuum.

The vacuum pump attached to the system is then started and reduction of pressure is first adjusted to approximately 0.5 atmosphere (380 mm. of mercury), at which it is maintained for 2 minutes. If the gas formation in the generating flask is then not sufficiently active, the pressure is further reduced to the point of optimum action, which, in the authors' experience is not below 0.25 atmosphere (190 mm. of mercury), and is held at this point for about 10 minutes, according to the activity of the gas formation. Pressure is then raised to the first level of 380 mm. of mercury and is kept at this point until 20 minutes in all have elapsed. During the run the temperature of the water bath should be from 15° to 20° C. Atmospheric pressure is restored to the system at the end of the run by gradually admitting air through the air-intake valve on the vacuum gage. It is convenient to have two or more units operating simultaneously on the same vacuum line (see arrangement shown in Figure 2).

Upon completion of the run, the filter disk bearing the spot reaction is removed from the clamping assembly and is immediately treated with 1 or 2 drops of the ammonia solution, which temporarily darkens the spot test to some shade of warm gray. The disk is now surface-dried by contact with clean filter paper and at once evaluated for arsenic content by comparison with a standard photographic step scale which has been calibrated to micrograms of arsenic as illustrated in Figure 4.

The degree of arsenic reaction may be read directly by placing the spot upon a white background and comparing its intensity with the shades of the photographic scale through windows which are punched in its banded portion. Anything which appears to be less than 0.04 microgram of arsenic in a 6.35-mm. (0.25-inch) spot test is considered a "blank". This corresponds to 0.01 microgram of arsenic in the 3.17-mm. (0.125-inch) spot test and is the limit of sensitivity which the authors have set for the method in routine practice. Incidentally, it is a severe test on laboratory technique and purity of reagents and in earlier experience there have been considerable periods of time when, owing to a lapse of purity in one of the reagents, it has been impossible to get satisfactory blank tests with the 3.17-mm. (0.125-inch) filtration area, or even with the 6.35-mm. (0.25-inch) area. Such lapses were, of course, definite departures from the tolerances given above under Reagents. The fault was usually found in the sulfuric acid and was accounted for by the fact that this reagent, although of the highest purity obtainable at the time, had been stored and marketed by the manufacturers in ordinary flint-glass bottles (which are not arsenic-free), and was probably also due to incidental air-contamination through use of too large containers. With the cooperation of the manufacturers this difficulty has been corrected and it is now possible regularly to get satisfactory blanks (less than 0.02 microgram) when operating with the 6.35-mm. (0.25-inch) filtration orifice. (The J. T. Baker Chemical Co. will now supply on request "acid sulfuric C.P. special arsenic-free" and "acid hydrochloric C.P. special arsenic-free" in 500 ml. glass-stoppered Pyrex bottles.)

In dealing with very small quantities of material the authors have encountered interferences from only two elements which may obscure the ammonia-treated Gutzeit end reaction. In rare instances of clinical blood examination following a recent intravenous injection of thiosulfate (administered to alleviate poisoning from overdose of a therapeutic arsenical), the hydrogen sulfide evolved in the generator has not been completely removed by the lead acetate scrubber. This occurred not in the volatile fraction, but in the residual fraction. Here, warning is given by a rapid and intense blackening of the scrubber, and the obvious remedy is to use a smaller sample or aliquot, which will still be adequate for determining arsenic concentration, which in these cases, is high. Occasionally, when samples of phosphatic urine have been evaporated, an excessive amount of volatile phosphorus has distilled over at 60° to 80° C. into the extraction train. In such cases, although the ammonia reagent does not darken the yellow stain caused by phosphorus, the ammonia-darkened reaction for arsenic will be degraded by the interference so as to cause false readings on the scale. Smaller aliquots, or removal of the phosphorus by distillation with hydrobromic acid as mentioned below, will prevent the difficulty.

Having never had interferences from antimony or selenium, the authors believe that, with the small samples of material needed for ultramicrodeterminations, this problem would arise only in exceptional circumstances, as in examining animal blood or excretions following ingestion of seleniferous or antimoniferous food.

In experiments with the addition of small amounts of sodium selenate to test samples of arsenical urine, they found that any concentration of selenium likely to occur—i.e., under 50 micrograms per 100 cc.—would be removed as selenium hydride along with hydrogen sulfide by action of the lead acetate scrubber. In the event of combined interferences, in cases of selenium or antimony poisoning complicated with arsenic poisoning of organic material, they have been prepared to work with relatively large samples and to remove phosphorus and antimony by distilling with 45% hydrobromic acid and an excess of bromine into the vacuum-extraction system, thus isolating arsenic with selenium in the distillate; then to separate selenium from arsenic by precipitating the selenium from the distillate with sulfur dioxide and hydroxylamine hydrochloride, according to the method of Robinson, Dudley, Williams, and Byers (*21*), leaving arsenic in the filtrate to be brought into a proper test solution for end determination by the present method, choosing an aliquot suitable to the ultramicro range.

PHOTOGRAPHIC STANDARDS FOR EVALUATION OF GUTZEIT SPOT TESTS

The first essential for producing a photographic step-scale print for the evaluation of Gutzeit spot tests is a primary film trans-

parency presenting a banded scale of light-transmission densities, arranged in a progressive exponential series, and in convenient dimensions for contact printing—as, for example, showing 21 contiguous bands covering a strip of film 20 to 22.5 cm. (8 to 9 inches) long and of convenient width, as 1.375 inches or 35 mm.

Such a primary step-scale transparency may be made on an optical bench by successive steps of equal time-unit exposure to a constant source of light and at progressive distances from the light, the precisely measured distances constituting a definite logarithmic series of steps to produce a corresponding logarithmic series of densities in the exposed photographic film after development. A special machine, such as the Eastman sensitometer, Type IIb, devised for testing the characteristics of photographic emulsions, will accomplish the same purpose. The authors produced their first films for photographic standards by the optical bench method, but they do not recommend it, since it not only is very laborious, but requires much technical skill and experience and faultless technique to get satisfactory results. They later procured from the Eastman Kodak Co. a number of photographic "step-tablet" transparencies made by their sensitometer machine. These step tablets are now commercially available for use in 3-color process work. One of these transparencies which was accurately calibrated at the Physics Department Research Laboratories of the Eastman Kodak Co., Rochester, N. Y., served to produce the secondary negative film referred to in Figure 5, and from which the step-scale prints illustrated in Figure 4 were printed.

This secondary or master-negative film is required, because the steepness of gradation of the original film is too great to serve directly for making prints which will have the finely graduated steps that are required in the final product.

A negative is therefore made by contact-printing from the primary transparency upon an 8 × 10 film having a rapid, long-scale emulsion, such as "Defender, XF-Panchromatic" or "Eastman, Tri-X Pan All", both of which emulsions possess a long straight-line portion in the low-development gradients of their characteristic (D-log E) curves. The aim here is to produce a negative step-scale film transparency as perfect as the original, but with its series of densities reduced in range while retaining the same number of steps as in the original—for example, from density 0.45 to density 2.41 in 21 steps as shown in Figure 5 (inset), a zone covered by only 14 steps of the original film.

This result is obtained by suitable exposure and development, so that the secondary negative, when calibrated by densitometer and plotted as a graph, will show a straight line similar to that of the original film, but with a lesser slope or gradient corresponding to a gamma of definitely shortened development. This task requires patience and considerable technical skill; but, once accomplished, there is no further difficulty and the resulting master negative is permanently useful for making contact prints on bromide paper to serve as standards for a variety of ranges of determination, as illustrated in Figures 4 and 5.

A suitable grade of bromide paper is required for making prints. It must be of pure white stock with a matte or rough surface. Its emulsion must be susceptible to full development without tendency to fog, and capable of giving a warm tone which will match the tinge of the ammonia-treated spot reactions. This is accomplished by adjusting the proportion of potassium bromide in the developer. "Defender, Velour Black, C-1", developed with "55D" formula diluted 33 to 50% to include addition of 10 to 20% of potassium bromide solution (10% by weight), has given good results. The time of exposure and development will vary slightly with different batches of paper. For instance, print B, Figure 4, was "VB-C-1" exposed 30 seconds at 85 cm. (34 inches) from a 60-watt Mazda lamp and developed in "55D" (12.5% potassium bromide solution added) at 65° F. for 50 seconds, the darkest band appearing at 22 seconds and the lightest at 49 seconds; print A, same paper and conditions, was exposed at 90 cm. (36 inches) from the lamp, and development was 28″/58″; print C was obtained by using a slightly "harder" grade of paper ("VB-C-2"). An acetic acid short-stop bath is used to stop development abruptly, and the print, after thorough rinsing in this, is fixed in two successive trays of plain "hypo" solution, 3 to 5 minutes in each tray with agitation. After a wash in running water for 20 to 30 minutes, the print should be treated to eliminate any thiosulfate which cannot be removed by washing, since residual traces as small as 1 microgram per sq. cm. of paper, will, in course of time, produce slight fading in the bands of lesser density due to a gradual "sulfiding" of the silver deposit. To correct this, the method of hypo elimination devised by Crabtree (*9*) is most reliable, a process which utilizes hydrogen peroxide and ammonia to oxidize every trace of thiosulfate for removal in a 5- to 10-minute period of final washing.

Even with these precautions, atmospheric conditions may cause degradation of the lesser densities, as, by hydrogen sulfide or sulfur dioxide in the air of industrial centers, or by salinity near the sea coast. A calibrated silver print should therefore be regarded as a delicate photometric standard and preserved carefully from the action of light, heat, and moisture; unless subjected to the sulfur-eliminative treatment recommended above, it should be recalibrated after 6 months. In any circumstances, a new standard print should be made from the master negative when a 12-month period has elapsed.

Prints are standardized by calibration against known amounts of volumetric arsenic solution as specified under Reagents. This is done by photometry, but expert visual calibration from known test spots, or from previously calibrated prints, will give

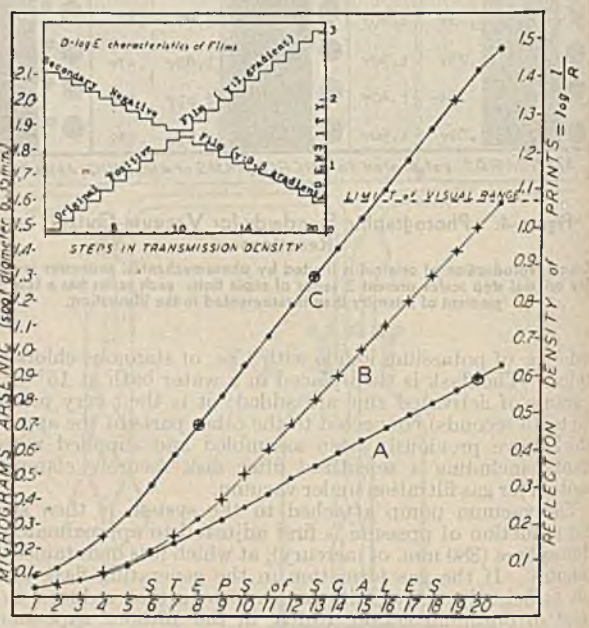

Figure 5. Reflection Densities of Photographic Scales

Step-scale prints A, B, and C with arsenic values coordinated to indicate system of calibration.

Dots are plotted against the right-hand ordinate of reflection density, expressed as log $\frac{I}{R}$, where I is the intensity of light reflected from the blank paper base, free of deposit (whether Ag deposit of the print, or As-Hg deposit of the test reaction), and R the intensity of light reflected from the deposit. The abscissa measures the D-log E characteristics of the developed bromide emulsion of the prints, in steps, as printed through corresponding bands of the negative film shown in the insert. The left-hand ordinate is an arithmetic progression, in terms of micrograms of arsenic as contained in Gutzeit test spots 6.35 mm. in diameter—a unit test area selected to establish two datum levels of 1.00 and 0.10 microgram of As, amounts which in repeated photometric evaluations coincided very closely with 0.666 and 0.066, respectively, on the reflection density scale, as indicated at steps 15 and 4 of the B-print graph. Dots occuring alone at step intervals in the graphs represent photometer allocations of the corresponding bands of prints and refer to the right-hand ordinate.

Crosses represent definite amounts of arsenic added to test solutions, and are the theoretical basis of calibration as referred to the left-hand ordinate. Dots which closely coincide with crosses are the actual coordinating factors plotted as calibrating factors by photometer readings of their reflection densities, and representing arsenic found, they are thus referable to both ordinates. Differences between crosses and nearly coincident dots, denote recovery data, or error of determination, and are summarized in the text as applying to different ranges. Dots marked with a ringed cross are duplications of primary calibrations on graph B. It is evident that this system of photographic standards for evaluation may be resolved to the formula: $\frac{a(RD)}{0.666}$, or, $1.5a(RD) = \gamma As$, where a is the unit area of 6.35 mm. diameter and RD the reflection density in terms of log $\frac{I}{R}$.

results which are surprisingly close to calibrations by galvanometer readings with a photoelectric cell. For photometric calibration the authors have employed the following apparatus:

A selenium photocell with a 20-mm. effective diameter, giving a photocurrent of 150 microamperes and output of 25 microwatts at 1 lumen. This is placed in an insulated adapter, or in a microphotometric slit ocular, at the eyepiece position of a microscope and connected with a mirror galvanometer of approximately 750-ohm resistance and a sensitivity of 2.5×10^{-9} ampere per mm., with the interposition of a compensating resistance circuit, so designed as to provide selective sensitivity with adjustable zero point on the galvanometer scale under a wide variation of light intensities or field areas.

A microscope fitted with 24-mm. and 40-mm. single-lens objectives, and with tube length adjustable from 155 to 200 mm. for controlling exact size of field, a mechanical stage for multiple (reduced field) readings, and a quick-shifting device for alternately positioning test spots and step-scale bands within the illuminated field of the instrument. (Precision of operation is favored by averaging or integrating fractional readings by means of a microphotometric slit ocular as described by Lange, *18*.)

An efficient type of electric microscope lamp and condenser, adjustable to an approximately 45° angle of incidence to prevent specular reflection, and capable of illuminating a field of 7 mm. or less in diameter. This lamp must be operated by a storage battery to ensure a nonfluctuating current and must produce a light of high intensity which is so steady as to cause no oscillation of the galvanometer light beam.

With such a system, the difference in photocurrent of reflectances from unit areas of adjacent bands of a step scale, such as scale *B* in Figures 4 and 5, will give deflections of the light beam on the galvanometer scale of 3 to 15 mm., as controlled by sensitivity adjustment in the balanced circuit. Reactions from known amounts of arsenic or from unknown test solutions can thus be nearly matched with scale-band deflection readings and values interpolated according to the step deflection.

The precision of the combined vacuum-Gutzeit procedure and photometric reading method, when summarized from the recovery data of Figure 5, shows: (*a*) in the lower part, or "toe" portion, of curves *B* and *C* (from 0.04 to 0.40 microgram of arsenic), comprising 7 recoveries, an accuracy of 95.85%, with a mean deviation of ±0.04 microgram; (*b*) in the range covered by the straight-line portion of the graphs (from 0.50 to 2.0 micrograms of arsenic), comprising 15 readings on 12 recoveries, an accuracy of 99.93%, with a mean deviation of ±0.0053 microgram; (*c*) an over-all accuracy, for the entire range, of 98.63%, with a mean error of ±1.37%. This applies to an exemplary set of findings and is somewhat better than the general average of ±1.5%, which is claimed for the photometric application of the method. For routine visual evaluations in the optimum range of 0.04 to 0.90 microgram of arsenic, precision is estimated as within ±0.03 microgram, and the visual error increases from ±0.06 to ±0.07 microgram in the higher range from 0.90 to the visual limit of 1.6 micrograms of arsenic, which implies a ±3% error in the optimum range.

Only the bands of reflection density on the calibrated scales are the essential criteria for arsenic determination, and these represent ascending gradients of arsenic value strictly according to the area of the test spot. When such a step-scale print has once been calibrated for the unit area of 6.35-mm. (0.250-inch) diameter, as shown in Figures 4 and 5, it follows mathematically that any step of the scale when referred to 3.17-mm. (0.125-inch) test spots, will represent just one fourth of the value attributable to the unit area. Thus, if an area be selected that is 2.5 times that of the unit area, or 10 times that of the 3.17-mm. area, its calculated area would be 79.167 sq. mm., its diameter 10.04 mm., and there would be established a triple ratio of 0.25:1:2.5 or 1:4:10, in scale value, according to spot area, with corresponding arsenic values holding throughout all shades of the scale.

SUMMARY

A relatively rapid procedure and new apparatus are described for isolation under vacuum of small amounts of arsenic as arsenic trihydride. The method is especially applicable to very small samples of fresh biological material and other organic substances which demand an ultramicro range of determination. It employs the well-known zinc-acid reduction process, which is shortened in time of operation to 15 minutes and intensified in action without applying heat. This is accomplished by operation in a vacuum system at 0.25 to 0.5 atmosphere to produce an arsine–mercuric bromide reaction by spot filtration. The reaction is concentrated by jet-filtration through a precisely gaged area of the sensitized medium. The diameters of the jets producing the spot reactions are designated as 3.17 mm. (0.125 inch) and 6.35 mm. (0.250 inch), giving two orders of reaction areas in the ratio 1 to 4, and providing ranges of 0.01 to 0.40 and 0.04 to 1.50 micrograms of arsenic, respectively.

These Gutzeit spot reactions, after intensification with ammonia, are immediately evaluated by reference to a standardized photographic step-scale print having 20 bands of mathematically graded reflection densities, which have been photometrically calibrated against fresh spot tests from known amounts of arsenic. The method of calibrating these photographic standards by coordination with recovery data, and the photographic procedure for producing the prints and the permanent film negatives from which they are derived, are described. Apparatus comprising the vacuum-filtration system consists mainly of parts which are precisely machined from methyl methacrylate polymer.

Sensitivity claimed for the method in its present form is 0.01 microgram of elementary arsenic, which is visually distinguishable from a "blank". Accuracy claimed for the vacuum-Gutzeit method combined with evaluation by photographic standards is ±3% by visual inspection in the range 0.02 to 0.90 microgram of arsenic and ±1.5% by photometry in the extended range to 2.0 micrograms of arsenic. Larger or smaller filtration areas may be used for other ranges than those described.

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Microchemical Identification of Demerol

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Demerol may be identified microchemically through formation of crystals with alkaloidal reagents. Doubly confirmatory test is available with single reagent, in conjunction with scratching of test drops. Tests with picric acid, lead iodide, sodium nitroprusside, potassium dichromate, and potassium iodide are described.

ALKALOIDS and alkaloidlike compounds, in general, combine with the so-called "alkaloidal reagents" to form insoluble complexes, many of which crystallize in unique and characteristic forms. Most of the natural alkaloids and many synthetic alkaloidlike compounds may be definitely identified by visual microscopic examination of these crystals. Several books and many articles describe or illustrate a number of those for which the specificity of the microcrystalline form has been established (*1*, *4*, *5*, *8* and others).

The usual procedure in making the microchemical tests is to add a drop of the reagent to a drop of a solution of the alkaloid on a glass slide; the test drop is then allowed to stand until crystal formation takes place. Rubbing or stirring the test drop to induce or hasten crystallization is, in general, considered to be objectionable. Stephenson (*6*) states that crystals formed on stirring are likely to be less characteristic than those formed more slowly, and recommends that stirring be avoided to prevent the formation of abnormal crystal forms. Scratching is sometimes recommended to induce crystallization (*2*), but no reference has been found in which scratching is used to influence the course of the crystallization.

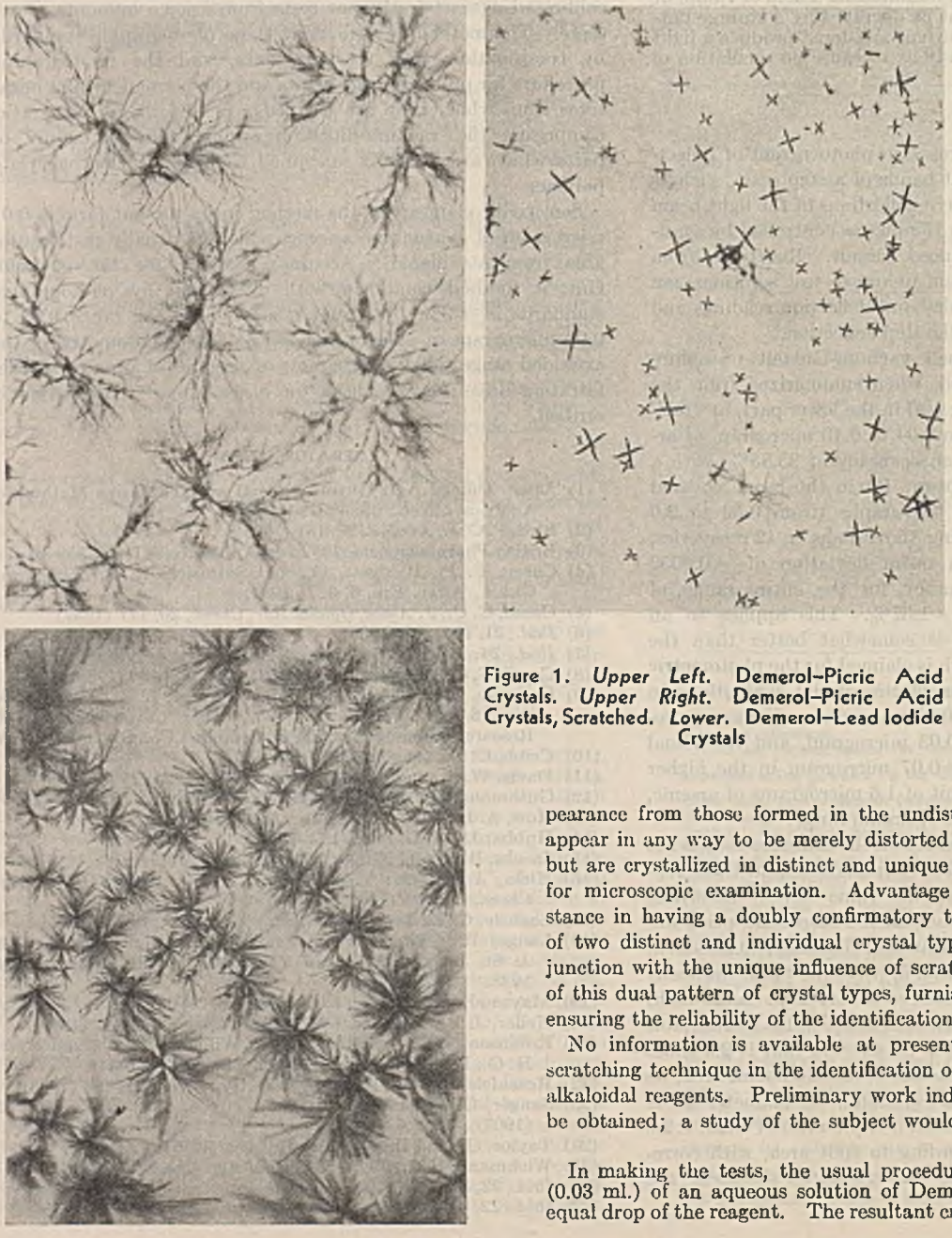

Figure 1. *Upper Left.* Demerol–Picric Acid Crystals. *Upper Right.* Demerol–Picric Acid Crystals, Scratched. *Lower.* Demerol–Lead Iodide Crystals

Demerol (ethyl-1-methyl-4-phenylpiperidine-4-carboxylate, *3*) may be readily identified microchemically. Its reaction with 28 of the common alkaloidal reagents was studied. Of these, 5 combined to form crystals which are very well formed and suitable for purposes of identification; 7 others formed crystals which, either because of low sensitivity or because of atypical crystal formation, are less suited for the purpose; 11 formed only amorphous precipitates; and 5 failed to form any precipitate.

The effect of scratching the test drops of Demerol and picric acid, sodium nitroprusside, or potassium iodide is very striking. Well-formed individual crystals are obtained which are entirely different in appearance from those formed in the undisturbed test drop. They do not appear in any way to be merely distorted or abnormal forms of the latter, but are crystallized in distinct and unique forms which are very well suited for microscopic examination. Advantage may be taken of this circumstance in having a doubly confirmatory test for Demerol: the formation of two distinct and individual crystal types with a single reagent, in conjunction with the unique influence of scratching in effecting the formation of this dual pattern of crystal types, furnishes a cross-combination of data ensuring the reliability of the identification.

No information is available at present as to the effectiveness of the scratching technique in the identification of other alkaloids with the various alkaloidal reagents. Preliminary work indicates that fruitful results might be obtained; a study of the subject would be of decided interest.

In making the tests, the usual procedure was employed. To one drop (0.03 ml.) of an aqueous solution of Demerol hydrochloride was added an equal drop of the reagent. The resultant crystals, formed either on standing

or on scratching with a glass rod, were examined under a magnification of 90×. Best results, in regard to facility of examination of the crystals, were obtained if the concentration of the Demerol solutions was such that not more than a slight initial precipitate was formed upon addition of the reagent. Hydrochloric acid, 0.1 *N*, may be used instead of water as solvent for the Demerol, except in the case of picric acid. Here the effect of the acid prevents the formation of the characteristic X-shaped crystals.

Crystalline complexes were obtained from the reaction of Demerol with picric acid, potassium iodide, sodium nitroprusside, lead iodide, potassium dichromate, potassium chromate, chromic acid (the latter three in hydrochloric acid solution), potassium ferrocyanide, potassium ferricyanide, platinic chloride, mercuric chloride, and palladium chloride. Amorphous precipitates only were obtained with gold chloride, Wagner's reagent, Marme's reagent, Mayer's reagent, phosphotungstic acid, phosphomolybdic acid, silicotungstic acid, zinc chloroiodide, sodium phosphate, sodium cobaltinitrite, and picrolonic acid. No precipitate was obtained with ferric chloride, zinc chloride, ammonium thiocyanate, saccharine, or potassium permanganate.

REAGENTS AND CRYSTALS BEST SUITED FOR IDENTIFICATION

Picric Acid, saturated aqueous solution.

This reagent is very sensitive. Best results are obtained with concentrations of Demerol of 0.1% or less. An amorphous precipitate forms, which is transformed on standing to large rosettes, the arms of which are very fine, wavy filaments (Figure 1, upper left).

If the reaction drop is stirred immediately after the addition of the solutions, X-shaped crystals form (Figure 1, upper right). With higher concentrations of Demerol, both forms of crystals may be found in the same test drop after scratching.

If the Demerol is dissolved in 0.1 *N* hydrochloric acid instead of water, no X-shaped crystals will form, even on scratching. Instead, there are formed, in addition to rosettes, long, very fine, needles. Addition of a very small amount of sodium bicarbonate to the Demerol solution promotes the formation of X's; if larger amounts of sodium bicarbonate are used, some crystals of this type may form even in the undisturbed drop.

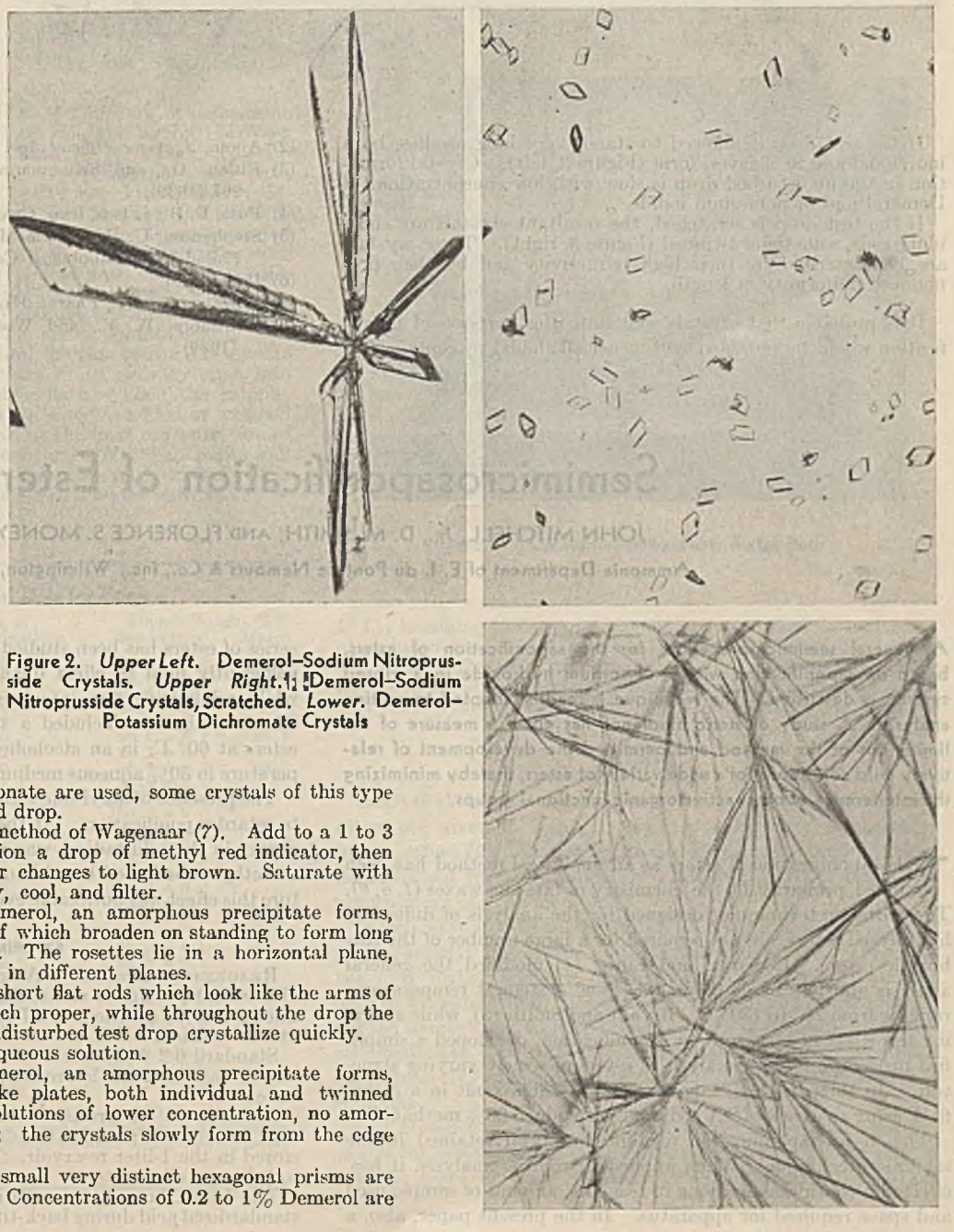

Figure 2. *Upper Left.* Demerol–Sodium Nitroprusside Crystals. *Upper Right.* Demerol–Sodium Nitroprusside Crystals, Scratched. *Lower.* Demerol–Potassium Dichromate Crystals

Lead Iodide, prepared by the method of Wagenaar (7). Add to a 1 to 3 aqueous potassium acetate solution a drop of methyl red indicator, then acetic acid until the yellow color changes to light brown. Saturate with lead iodide while warming gently, cool, and filter.

With a 0.1% solution of Demerol, an amorphous precipitate forms, changing to rosettes, the arms of which broaden on standing to form long flat plates (Figure 1, lower left). The rosettes lie in a horizontal plane, and overlie other rosettes lying in different planes.

If the test drop is scratched, short flat rods which look like the arms of the rosettes form along the scratch proper, while throughout the drop the same types of rosettes as in the undisturbed test drop crystallize quickly.

Sodium Nitroprusside, 5% aqueous solution.

With a 1% solution of Demerol, an amorphous precipitate forms, changing to long coarse bladelike plates, both individual and twinned (Figure 2, upper left). With solutions of lower concentration, no amorphous precipitate forms initially; the crystals slowly form from the edge of the drop.

If the test drop is scratched, small very distinct hexagonal prisms are formed (Figure 2, upper right). Concentrations of 0.2 to 1% Demerol are suitable. In accord with the orientation of the prisms, the hexagons may or may not appear equilateral. The relative lengths of the sides may be such that the crystals appear rhomboidal or, sometimes, diamond-shaped.

Potassium Dichromate–Hydrochloric Acid. A 5% solution of potassium dichromate in a mixture of equal parts of concentrated hydrochloric acid and water.

An amorphous precipitate forms, changing to a dense mat of long yellow needles, often in sheaves (Figure 2, lower left). With concentrations of Demerol under 0.2%, no amorphous precipitate is formed; direct crystallization takes place slowly. Scratching hastens the crystallization, without affecting the crystal form.

Similar crystals are formed with potassium chromate or chromic acid in hydrochloric acid solution. Neutral aqueous solutions of the three reagents produce only amorphous precipitates.

Potassium Iodide, 20% to saturated aqueous solutions.

The sensitivity of this reagent varies with its concentration. A 20% solution is sensitive to a 0.2% Demerol solution, while sensitivity to below 0.05% Demerol solutions may be obtained by saturating a test drop of the latter with crystalline potassium iodide.

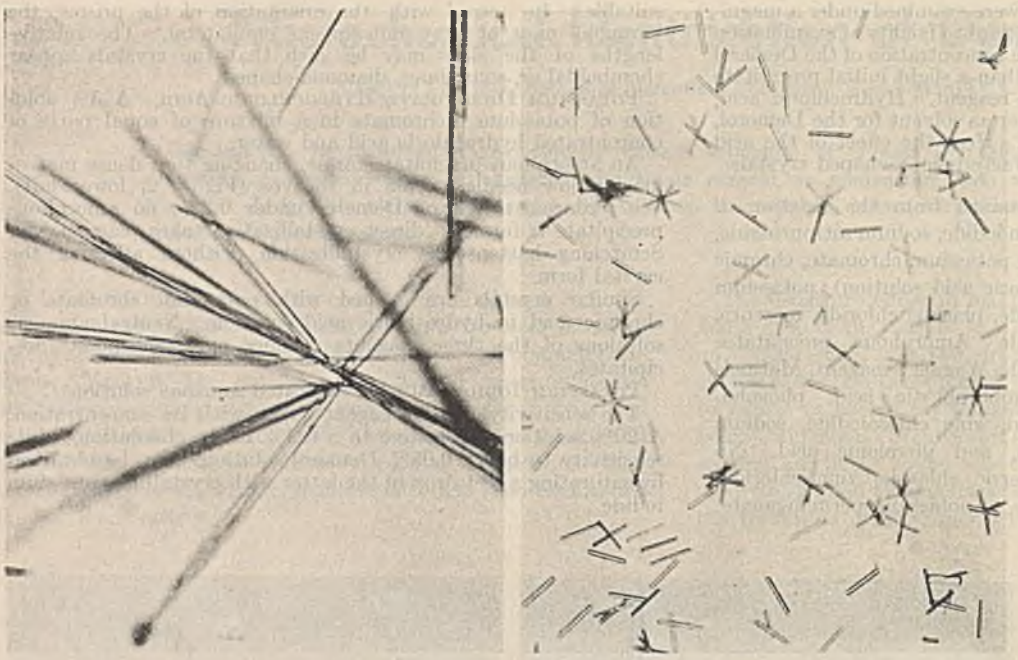

Figure 3.
Left. Demerol–Potassium Iodide Crystals.
Right. Demerol–Potassium Iodide Crystals, Scratched

If the test drop is allowed to stand, very long needles, both individual and in sheaves, form (Figure 3, left). Crystal formation in the undisturbed drop is slow with low concentrations of Demerol and of potassium iodide.

If the test drop is scratched, the resultant crystals are short blunt rods, sometimes twinned (Figure 3, right). These crystals are characterized by their high refractivity and by their pronounced uniformity in length.

It is probable that crystals well suited for purposes of identification would be obtained with other alkaloidal reagents. The reagents used, however, are common, and the above descriptions provide adequate data for the unequivocal identification of Demerol.

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Semimicrosaponification of Esters

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A general semimicroprocedure for the saponification of esters, based on saponification with 2 *N* sodium hydroxide in a closed system, is described. The technique is readily adapted to routine analysis. A study of steric hindrance has given a measure of the limitations of the method and permitted the development of relatively mild conditions for a wide variety of esters, thereby minimizing the interference of other active organic functional groups.

THE saponification of esters as an analytical method has been studied primarily in the chemistry of fats and waxes (*1*, *5*, *6*). These drastic techniques, designed for the analysis of difficultly hydrolyzed esters, are not required for a large number of the carboxylic esters. Redeman and Lucas (*4*) modified the general analysis of esters by employing a closed system at temperatures ranging from 70° to 130° C. Bryant and Smith (*2*), while studying the effect of structure on saponification, developed a simplified procedure for the analysis of esters of widely varying structure. In their method, saponification is carried out in a closed flask using an excess of 2 *N* sodium hydroxide in 90% methanol.

This basic saponification medium has been retained in the semimicrotechnique. When applied to routine analysis, it has effected a considerable saving in reagents, amount of sample used and space required for apparatus. In the present paper, also, a series of esters has been studied in order to determine conditions sufficiently mild to effect quantitative hydrolysis of the esters while minimizing the interference of aldehydes and ketones. The investigation included a study of relative activity of the esters at 60° C. in an alcoholic environment and at room temperature in 50% aqueous medium.

The presence of alkyl side chains near the ester groups is known to retard saponification. At the higher temperature α-alkyl hindrance in the acid radical was particularly marked between the α-methyl-substituted butyl and amyl esters. At room temperature this effect was noticeable even in the simplest esters.

EXPERIMENTAL

Reagents. Alcoholic 2 *N* sodium hydroxide is prepared by dissolving 80 grams of c.p. sodium hydroxide and 100 ml. of distilled water in sufficient Du Pont synthetic methanol to make 1 liter of solution.

Standard 0.2 *N* hydrochloric acid is prepared by diluting 19.8 ml. of concentrated hydrochloric acid to 1 liter with distilled water.

Apparatus. A precise vacuum filling pipet (Figure 1) is used to deliver reproducible volumes of the 2 *N* caustic, which is stored in the 1-liter reservoir. The apparatus is protected with soda lime at all outlets.

The 10-ml. buret (Figure 2) is used to deliver accurately the standardized acid during back-titration. (These items were made

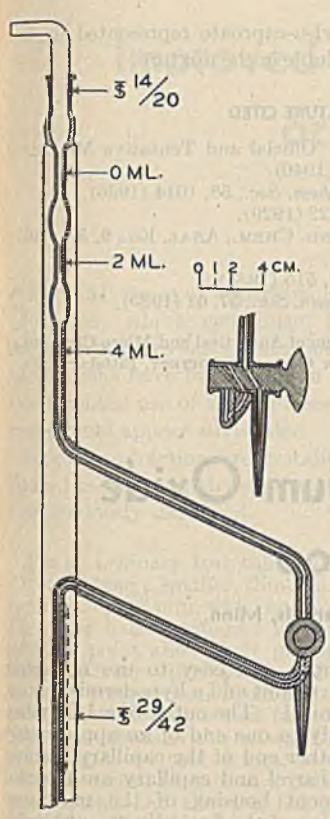

Figure 1. Delivery Pipet

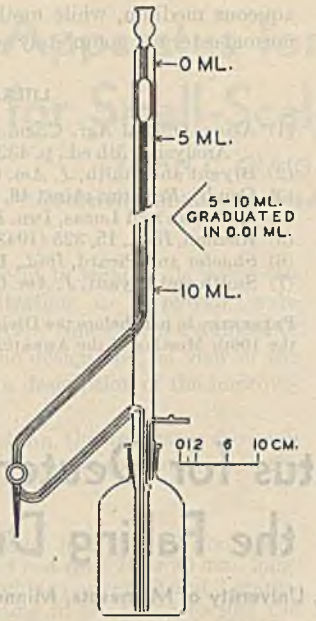

Figure 2. Microburet

to the authors' specifications by Eck and Krebs, 131 West 24th St., New York, N. Y.)

A convenient water bath (Figure 3), initially described in an earlier publication (*7*), has been converted to a thermostatically controlled unit. Equipped with Arthur H. Thomas Co. 60-cm. (24-inch) clamp-bars and No. 3237 G, size 3C, micro-spring-grip clamps, one bath will accommodate up to twenty 25-ml. or 50-ml. flasks, simultaneously.

Analytical Procedure. A 2-ml. portion of the 2 *N* caustic solution is delivered from the vacuum filling delivery pipet into a 25-ml. glass-stoppered volumetric flask. Then the sample, containing up to 2 milliequivalents of ester, is added or weighed into the flask. In the general analysis the final concentration of sodium hydroxide should not be less than 1 *N*. This limits to 2 ml. the total volume of sample or sample plus diluent. The flask, together with a blank, is placed in a water bath at 60° ± 1° C., loosening the stopper momentarily to allow for expansion of included air. Then the flask is stoppered firmly, heated for 30 minutes, removed from the bath, and cooled in ice water. Excess alkali is determined by back-titration with the standardized 0.2 *N* hydrochloric acid to the phenolphthalein end point.

Table I. Analytical Data for Esters

Ester	No. of Determinations	Found, Weight % Semimicro	Macro
Methylacetate[a]	2	99.0 ± 0.0	...
Ethylacetate[b]	2	98.7 ± 0.2	...
Monoacetin[c]	2	105.6 ± 0.3	...
Isopropylacetate	2	98.8 ± 0.0	...
Diacetin[c]	2	92.3 ± 0.2	...
Ethylpropionate	6	99.7 ± 0.2	99.9
Triacetin	2	99.2 ± 0.1	...
n-Butylacetate	10	98.8 ± 0.2	98.7
Isobutylacetate	2	97.0 ± 0.1	...
tert-Butylacetate[d]	2	98.3 ± 0.2	...
Ethyl-*n*-butyrate	2	100.2 ± 0.0	...
Ethylisobutyrate	2	97.9 ± 0.2	...
Methyl-*n*-valerate	4	98.8 ± 0.2	99.0
Methyl-*n*-caproate	2	98.8 ± 0.2	...
Methyl-2-methylpentanoate[e]	2	67.3 ± 0.0	...
Ethyltiglate[e]	2	100.0 ± 0.0	...
Ethyl-2-methylbutanoate[e]	2	92.3 ± 0.0	...
Isobutyl-*n*-butyrate	4	98.5 ± 0.2	98.5
Isobutylisobutyrate	2	99.7 ± 0.1	...
Ethyladipate	6	99.2 ± 0.1	99.2
Ethylethylmalonate	2	92.8 ± 0.1	...
n-Amyl-*n*-caproate	2	93.2 ± 0.2	...
Isobutyladipate[e]	4	100.2 ± 0.1	100.2
Cyclohexylacetate	2	100.2 ± 0.2	...
Cyclohexylisobutyrate[e]	4	97.9 ± 0.2	...
Cyclohexyladipate	4	99.9 ± 0.2	100.0
Ethylphenylacetate[f]	2	99.7 ± 0.1	...
n-Butylphthalate	2	98.5 ± 0.0	...
Benzylbenzoate	4	100.1 ± 0.1	...

[a] Carbide and Carbon Chemical. [b] J. T. Baker. [c] Eimer & Amend. [d] Prepared from *tert*-butanol and acetyl chloride in presence of pyridine (*6*). [e] Prepared from corresponding acid and alcohol. [f] Newport Chemical Works. All others Eastman chemicals.

Table II. Saponification of Esters in 30 Minutes at Room Temperature

Ester	Per Cent Saponified 2 *N* NaOH in 90% CH_3OH	2 *N* aqueous NaOH
Methylacetate	91.5	100.0
Ethylacetate	94.0	100.0
Monoacetin	...	100.0
Isopropylacetate	91.0	99.5
Diacetin	...	100.0
Ethylpropionate	91.5	100.0
Triacetin	...	100.0
Isobutylacetate	95.0	100.0
tert-Butylacetate	...	76.0
Ethyl-*n*-butyrate	...	99.5
Ethylisobutyrate	69.5	100.0
Methyl-*n*-valerate	...	100.0
Methyl-*n*-caproate	...	84.5
Ethyl-2-methylbutanoate	...	50.0
Methyl-2-methylpentanoate	...	40.0
Ethyltiglate	...	98.0
Isobutyl-*n*-butyrate	...	34.5
Isobutylisobutyrate	...	50.0
Ethylethylmalonate	...	92.0
Ethyladipate	...	100.0
n-Amyl-*n*-caproate	...	5.0
Cyclohexylacetate	...	100.0
Cyclohexylisobutyrate	...	6.0
Ethylphenylacetate	...	100.0
n-Butylphthalate	...	6.0
Benzylbenzoate	...	7.5

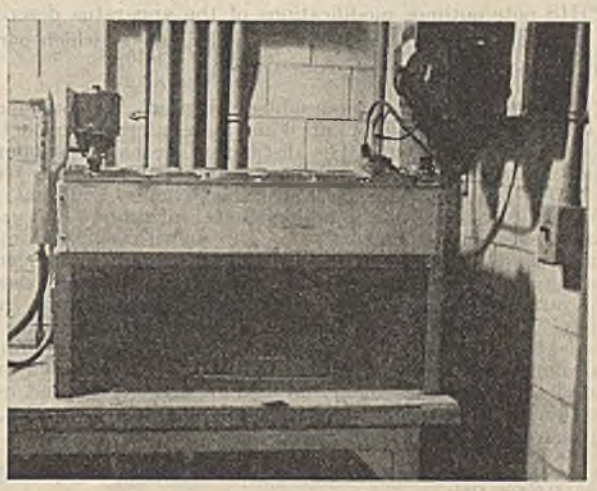

Figure 3. Constant-Temperature Water Bath

RESULTS

Analytical results obtained on twenty-nine widely different esters are given in Table I, together with some comparative macro values (*2*). Except where noted the trade products were used without further purification. In general, the precision and accuracy are each about ±0.2%.

Interference due to α-alkyl substitution was noticed first with ethyl-2-methyl butanoate and ethylethylmalonate. However, the next higher homolog, methyl-2-methylpentanoate, reacted only to the extent of 67%, a decrease of about 25%. By increasing the temperature to 100° C. and heating for 2 hours the ethylethylmalonate and ethyl-2-methylbutanoate were saponified quantitatively, while the methyl-2-methylpentanoate was 95% complete.

The presence of water in the sodium hydroxide solution is desirable for saponification in alcoholic reagent (*3*). In the general analysis, however, water in excess of 10% decreases the solvent

action of the methanol. These factors were verified during 30-minute room temperature studies, where the ester in methanol solution was added in 2-ml. portions either to 2 ml. of 2 *N* sodium hydroxide in 90% methanol or to 2 ml. of 2 *N* aqueous caustic. In the one case this represented essentially alcoholic environment and in the other about a 50% aqueous medium. Results obtained on several esters are given in Table II.

A comparison of results from the two reagents definitely proves the beneficial effect of water. In the alcoholic environment saponification was incomplete, even with the simplest esters. In the aqueous alcoholic medium, however, saponification was complete for most of the normal lower esters. *tert*-Butyl acetate showed the first marked evidence of side-chain interference in the aqueous medium, while methyl-*n*-caproate represented the first normal ester not completely soluble in the mixture.

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Modifications of Apparatus for Deuterium Oxide Determination by the Falling Drop

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THIS note outlines modifications of the apparatus described by Keston, Rittenberg, and Schoenheimer (*2*) which permit greater speed of deuterium oxide analysis.

Two distillation trains are used (see diagram), the components of which are more readily cleaned and more interchangeable than those of Keston *et al.* The distillation trains are supported rigidly only at positions *i*. Where many determinations are to be made, the following glassware will be found sufficient: 10 tubes *a;* 3 each of tubes *b* and *c;* 2 each of tubes *d* and *g;* 14 plugs *e;* and 6 weighing bottles *f*. Tubes *g* with weighing bottles can be attached to tube a_2 in place of the plug when the sample contains no organic material and combustion is unnecessary. The outlet of the combustion furnace is adapted to fit the *a* tubes. The sample, with barium carbonate, is boiled for a few moments before the pressure is reduced, to eliminate bumping. Calcium oxide is used in tube *b* in place of potassium hydroxide and chromic oxide to eliminate replaceable H^+. As condensing medium an ice-saturated calcium chloride bath is convenient, inexpensive, and entirely satisfactory.

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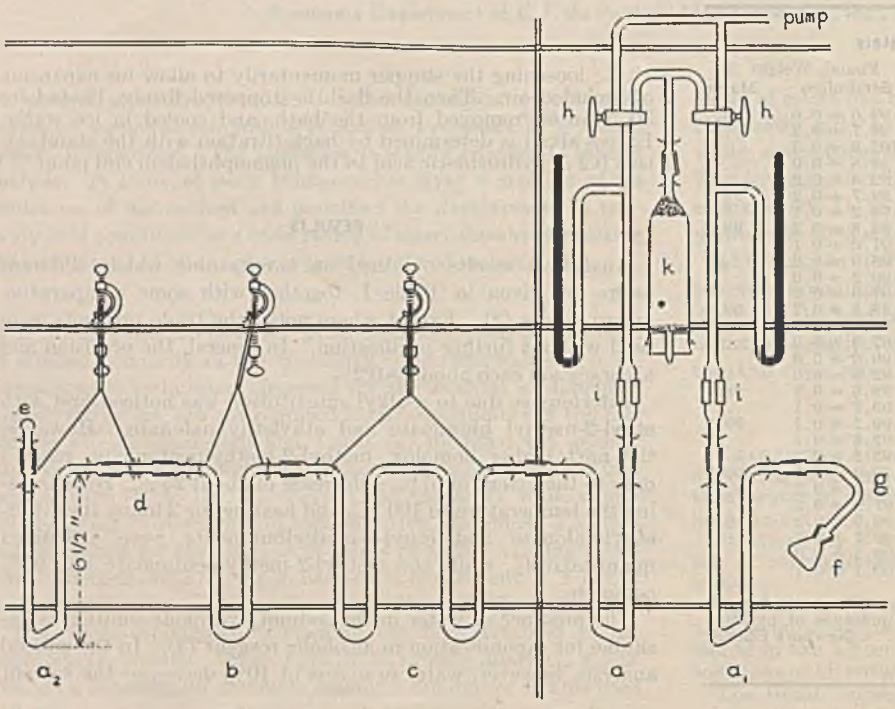

An accurate, relatively simple, and easy to use micropipet can be made from a microscope mount and a hypodermic syringe; its operation is entirely mechanical. The outlet of a 1-cc. tuberculin syringe is attached rigidly to one end of an appropriately bent 0.5-mm. capillary; the other end of the capillary is drawn to a small tip. The syringe barrel and capillary are mounted firmly on the coarse-adjustment housing of the microscope mount in such a way that motion of the fine-adjustment housing is not interfered with. A spring is slipped over the lubricated plunger of the syringe before it is inserted into the barrel. The plunger is actuated by a precisely threaded pin (from an inexpensive micrometer caliper, for example). The threaded sleeve in which this pin turns is attached to the fine-adjustment housing. Two stops allow the pin to be turned through only part of a revolution. The capillary and syringe are filled, under vacuum, with mercury. The coarse adjustment of the microscope mount raises and lowers the entire pipet; the fine adjustment is used for filling and flushing the capillary; rotation of the threaded pin through its prescribed limits delivers the droplet. The capillary may be provided with a removable tip for easy cleaning, and with a mercury reservoir.

Extremely vigorous stirring of the constant-temperature bath renders the maintenance of the required constancy relatively easy; such stirring may be achieved with little vibration with the impeller described by Hemingway and Shelley (*1*). As a thermoregulator, copper tubing filled with 1,2-dibromomethane has given excellent results; 1,2-dibromomethane has a low specific heat, high thermal conductivity, and high expansion coefficient, so that it is about twice as effective as the liquids usually used in thermoregulators. Ordinary cements cannot be used with this substance to attach the copper tube to the glass thermoregulator head; it should be sealed directly to the copper, or the glass tubing can be inserted into a cup of Wood's metal surrounding the copper tube. The heater element used is enameled copper wire connected to the secondary (10 volts, 12 amperes) of a transformer; this element has almost no heat lag. A mercury, constant-head overflow column is used to maintain a constant flow of cooling water through the bath.

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Improved Apparatus for Solubility Determination or for Small-Scale Recrystallization

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SEVERAL types of apparatus useful in small-scale recrystallization, where centrifuge filtration is employed, were described in a previous publication (*1*). Since then, a number of modifications have been made in the design and, in view of the wide potential use of such devices, a description of the improvements would appear advisable.

For routine fractional recrystallization, the apparatus shown in Figure 1 is easily constructed, and is considerably superior to the ones previously described.

A is an ordinary test tube, 100 mm. long and 12 mm. wide. *B* is made from a smaller, thin-walled test tube 70 or 80 mm. long, approximately 8 mm. wide, and weighing between 2 and 3 grams. The upper half is widened by heating in the flame to just the softening point and slowly pushing it over a hot carbon rod a little over 8 mm. in diameter. By steadily turning the tube on the rod, a very uniform shoulder having an angle of about 45° can be obtained. Another test tube of the same size will then just barely pass through the enlarged part. *C* is made by heating a test tube 8 mm. in diameter with a uniformly round bottom at a point about 15 mm. from the end and allowing the glass to collapse. When enough solid glass has collected at this point, it can be drawn out to form a solid rod 2 to 2.5 mm. in diameter, as shown in Figure 1, and approximately 60 mm. long. The lower end of the rod can be enlarged as shown, by the use of a flat carbon while the tip is molten. This gives the rod more strength so that it can better withstand the pressure during centrifuging. The combined weight of *B* and *C* should be of the order of 4 to 6 grams. *D* is a larger test tube which is fitted with a rubber stopper and has cotton on the bottom at *E*.

In actual practice, the material to be recrystallized is placed in the modified test tube, *B*, and solution is achieved with a minimum volume of hot solvent. The apparatus is then assembled in a position the reverse of that shown in Figure 1, and cooled to the optimum temperature for crystallization. After crystallization, it is inverted to the position shown and centrifuged. (A speed of approximately 1500 in a No. 2 International centrifuge has been found to give clear-cut filtration without breakage of the equipment.)

The crystals are caught at the point of enlargement of test tube *B*, since there is just enough clearance between *C* and the shoulder of the enlargement of *B* to allow the liquid but not the crystals to pass through. For fine crystals, *C* may be seated a little more accurately on the shoulder by grinding with rough Carborundum, as described below. After filtration, the inner test tube, *A*, is first removed and held at an angle of about 45°. *B* and *C* can then be removed as one piece by grasping the exposed end of *B*, since in this position *C* will bind in the larger part of *B* and will not fall out.

It is convenient to have *B* and *C* tared together, so that the weight lost during crystallization, the wet weight, etc., can be obtained. After filtration, *C* can serve as a stopper to prevent the entrance of extraneous material during storage or during further crystallizations or manipulations. When properly seated by grinding, the unit is entirely adaptable to solubility microdeterminations, as carried out by Ing and Bergmann (*2*) and more recently in a greatly improved way by Moore and Stein (*3*).

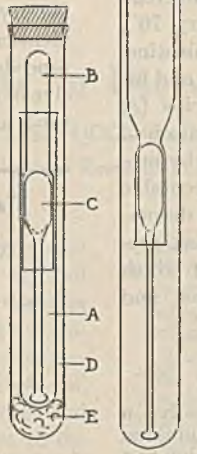

Fig. 1

Fig. 2

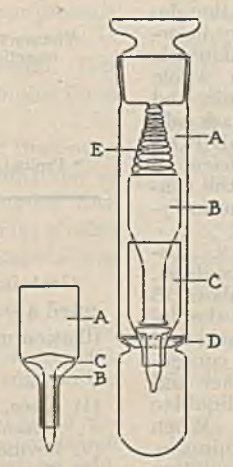

Fig. 3

Fig. 4

A slight modification in the design adapts the apparatus for use with much larger amounts of material. This is done by enlarging the crystallization part of *B* by blowing it larger from the same test tube, as shown in Figure 2, or otherwise making a very thin-walled bulb. The bulb or crystallization part in this case must be very thin, so as not to increase the total weight of the crystallization vessel and the filter stick much beyond 6 grams. Bulbs holding from 15 to 20 ml. have been constructed from this weight of glass and successfully used by starting the centrifuge a little more slowly. This volume permits recrystallization from 10 to 15 ml. of solvent.

For small-scale work, sintered filters are unquestionably useful, both for suction and centrifuge filtration, but are not always easy to clean, and this gives rise to a reasonable distrust of their reliability. They also retain appreciable amounts of mother liquor, and complete removal of the crystals from the rough surface without scratching off glass particles at the same time is generally difficult. An attempt to devise an all-glass filtration apparatus free from these objections has met with success in the filter shown in Figure 3, which is an improvement over the one previously described (*1*) (Figure 2).

A is a glass funnel of the shape indicated. The narrow part of the funnel is made from glass tubing approximately 5 mm. in inside diameter, and is approximately 15 mm. long. *B* is made by stamping out a molten glass rod or tubing with the appropriate carbon surface, so that it is approximately the shape shown and then grinding it to fit more accurately. The narrow part of funnel *A* acts as a sleeve to hold *C* in position, so that the two surfaces at *C* may be ground with 120-mesh Carborundum just to the point where the funnel, when in position, will completely remove fine bone black from an aqueous suspension.

Such filters, properly prepared, give surprisingly rapid filtration when the ground surface of *B* is not too long. Therefore, the ground edge of *B* at the point *C* is rather thin (1 mm. or less). They are effective even with the finest bone black. They may readily be taken apart for cleaning and are free from the objections of sintered filters. They are particularly useful for micro work and allow filters of very small diameter to be constructed. The principle involved is fundamentally the same as that with sintered filters, except that here the walls of the pores which effect filtration may be taken apart for cleaning.

For solubility determinations, the authors have modified the excellent apparatus of Moore and Stein (*3*), as shown in Figure 4, for use with this type of filter.

Tube *A* is exactly as described (*3*), except that a ground-glass stopper is substituted for rubber. The stopper is hollow and has a wide, flat top, so that the inverted tube will stand on the stopper during the necessary manipulation. Two small indentations are on opposite edges of the flattened top for rubber bands to hold tube in the equilibrating apparatus. The inside surface of the stopper has a button on it to hold the flexible wire

spring, *E*, which in turn holds the filtration apparatus and filter stick in place. Flask *B* is of very thin glass, for its rests against the filter stick to hold it in place and a heavy flask would break during centrifugation. Filter *C* is as described above, except that the lower part is as short as possible. It rests on a soft tin collar, *D*.

Using this assembly, accurate solubility determinations may be made with organic solvents or corrosive solvents, since no rubber is present to absorb any portion of the solvent.

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Semimicrodetermination of Arsenic in Insecticides

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THE procedure presented below represents an extension to insecticidal arsenicals of the method recently described (*4*) for the semimicrodetermination of arsenic in organic compounds, the arsenic being precipitated as element by action of hypophosphorous acid and determined iodometrically with the aid of Koppeschaar's bromide-bromate solution. This method is believed to be applicable to any properly prepared solution of arsenic free from interfering substances, such as organic material or metals precipitatable by hypophosphorous acid. It was found that acid-soluble arsenicals (Paris green, lead arsenate, calcium arsenate) could be analyzed thus, following dissolution of the samples in aqueous hydrochloric acid.

In presence of organic material, a preliminary decomposition similar to that described for the analysis of organic arsenicals (*2*, *4*) may be necessary. This was the case with the commercial insecticide currently marketed under the name "Victory 76", stated to contain calcium arsenate together with sulfur, nicotine, organic compounds with carbon contents from C_{10} to C_{18}, and inert material. Decomposition by nitric and sulfuric acids (*4*) was shown to be a suitable preparation for the determination of arsenic in this insecticide. An alternative decomposition by bromine was found to be more rapid and to lead to acceptable results, but is judged to be less satisfactory because the decomposition liquid contained suspended dark-colored material, presumably organic bromination products, the presence of which interfered visually at the time the arsenic was reduced and precipitated.

PROCEDURES

Substances Soluble in Hydrochloric Acid. Dissolve a weighed sample (0.5 to 2 grams) of dried material in a minimal volume of 6 *N* to 12 *N* hydrochloric acid, transfer the solution to a 500 ml. volumetric flask, and dilute to the mark. Transfer an aliquot portion containing about 15 mg. of arsenic to the flask of an all-glass decomposition apparatus with reflux tube, such as that described for use in the determination of arsenic or mercury in organic compounds (*3*, *4*). If a sufficiently sensitive balance is available—e.g., a semimicrobalance—weigh out the whole sample, of such size as to contain about 15 mg. of arsenic, and dissolve in hydrochloric acid. To the solution in the flask add and dissolve rapidly 3 grams of sodium hypophosphite ($NaH_2PO_2 \cdot H_2O$), and then add concentrated hydrochloric acid sufficient to increase the acid concentration to about 6 *N*. Attach the condenser and heat the flask with a small flame, completing the analysis as described (*4*).

Arsenical Mixtures Containing Organic Matter. *Decomposition by Nitric and Sulfuric Acids (recommended procedure).* Weigh accurately a sample of suitable size (to contain about 15 mg. of arsenic; 0.5 gram of Victory 76) and transfer to the decomposition flask. Add 25 ml. of concentrated nitric acid and warm the mixture for several minutes. Add 20 ml. of concentrated sulfuric acid, evaporate the mixture to fumes, then add more nitric acid and again evaporate to fumes. Allow the liquid to cool partially and introduce 1 gram of ammonium sulfate. When evolution of gas ceases, heat the liquid gently for 5 minutes. Cool, add about 50 ml. of water, and heat until the solution clears or is slightly opalescent. Add 35 ml. of concentrated hydrochloric acid, then 3 grams of sodium hypophospite, and continue as described (*4*).

Decomposition of Victory 76 by Bromine. Transfer weighed sample to the decomposition flask, add 2 ml. of liquid bromine, and swirl the mixture for about 5 minutes. Add 50 ml. of 6 *N* hydrochloric acid and heat moderately until nearly all the excess bromine is expelled (hood). To the cooled solution add 10 ml. of concentrated hydrochloric acid and 3 grams of sodium hypophosphite, and complete the analysis as indicated above.

RESULTS

Analytical results for the four materials mentioned are presented in Table I, which includes also comparative results obtained by the familiar distillation procedure (*1*) selected as an umpire method.

Discussion. Results by the reduction method show satisfactory levels of precision and accuracy, and are substantially identical with results by the distillation method. The reduction procedure is the more rapid, requiring about 40 minutes (exclusive of any needed preliminary decomposition), as compared with the 2 to 3 hours required for the distillation procedure.

Table I. Determination of Arsenic in Some Insecticides

Material	Arsenic Found: Reduction method	Distillation method
	%	%
Paris green	42.81	42.75
	42.77	42.72
	Av. 42.79	42.57
		Av. 42.68
Lead arsenate	20.25	20.23
	20.18	20.27
	20.15	20.30
	Av. 20.19	Av. 20.27
Calcium arsenate	26.65	26.84
	26.72	26.77
	26.73	Av. 26.81
	Av. 26.70	
Victory 76 insecticide	HNO_3–H_2SO_4 decomposition	Bromine decomposition
	3.57	3.64 3.62[a]
	3.50	3.55 3.57[a]
	3.60	3.60 Av. 3.60
	Av. 3.56	3.59

[a] Preliminary decomposition by HNO_3-H_2SO_4.

ACKNOWLEDGMENT

Grateful acknowledgment is made to J. J. McGlynn, who executed a series of confirmatory analyses by the reduction and distillation methods.

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NOTES ON ANALYTICAL PROCEDURES

A Vacuum Stopcock Lubricant Unaffected by Hydrocarbons

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DESPITE the frequent need in vacuum work for a stopcock lubricant that is unaffected by hydrocarbons, an inexpensive, readily prepared lubricant does not seem to be described in the literature. The glycol citrate polymer of Sager (*1*) is satisfactory towards hydrocarbons, but its viscosity characteristics make it unsuitable for vacuum work. After a few hours the lubricant flows out of the ground surfaces, and rapid leakage results. From analogy with the common rubber-paraffin lubricant, it appeared that a high-molecular-weight filler might add sufficient body to overcome this difficulty. The addition of cellulose acetate to the glycol ester produced a lubricant that is quite unaffected by hydrocarbons and that permitted the maintenance of a vacuum of 10^{-4} mm. of mercury after more than 6 months' use.

A solution of cellulose acetate was prepared by heating 7.5 grams of Celanese, cut into small pieces, in 45 grams of tetraethylene glycol. After 4 hours at 140° C., with frequent stirring, the solution appeared homogeneous. Citric acid (30 grams) was heated on an oil bath to 190° and the cellulose acetate solution added. Heating was continued at 180–190° C. for 90 minutes.

In order to remove dissolved water, the solution was immediately poured into a previously heated glass jar in a desiccator and the desiccator evacuated as rapidly as foaming permitted. The dehydration has little effect on the final consistency.

Stopcocks lubricated with this material showed no signs of failure after 6 months in contact with liquid toluene, repeated evacuation, and frequent turning. No changes in the properties of the lubricant were observed after storage in a closed jar for a year.

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Stopcock Lubricants for Use with Organic Vapors

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RECIPES for lubricants which are insoluble in most organic solvents have been suggested (*1–4*). Generally, glycerol has been used as a base while bentonite, dextrin, or dextrin and mannitol serve as thickening agents. While these undoubtedly behave well under ordinary conditions, sometimes the use of such a lubricant is required in a low-pressure system, where conditions are more exacting. Both bentonite and dextrin are difficult to dehydrate and dextrin is very apt to decompose when heated to the temperature necessary to disperse it thoroughly in glycerol. This is undesirable, since water is one of the decomposition products, and while the vapor pressure of anhydrous glycerol is about 4×10^{-4} mm. of mercury at 25° C., it rises to 0.4 mm. when it contains 2% water. This means that in order to obtain low pressures, long pumping times are necessary.

In an attempt to obviate this difficulty several other thickening agents were tried. The most successful were combinations of mannitol or crystalline carbohydrates such as sucrose with polyvinyl alcohol of medium viscosity. The polyvinyl alcohol itself made a good thickening agent, but the extra material was required to give the necessary film strength for lubrication.

Before making up, all materials were dried in vacuo at 70° to 80° C. This treatment concentrated glycerol from 94% to better than 99% in about 4 hours and a McLeod gage on the system showed a pressure of 10^{-4} mm.

Perhaps the most successful lubricant contained 1 to 3% of medium viscosity polyvinyl alcohol and 15 to 20% of mannitol, in glycerol. After the ingredients had been pasted in the cold, the mixture was carefully heated to about 130° C. and held there with continuous stirring until the dispersion was uniform and complete. Stirring, when crystals first appeared after the mix cooled, was beneficial in keeping the mannitol finely divided. Although the product was rather dry in appearance, it behaved well after repeated turning of the stopcock.

The mannitol may be replaced with about 40% of sucrose. This preparation behaves well without the polyvinyl alcohol. Sucrose crystals will usually appear in the supersaturated solution after about two days' standing, and stirring for a short time will keep them in a fine state of division.

With either of these lubricants no difficulty was experienced in obtaining and holding a McLeod gage pressure of 10^{-5} mm. They have been used successfully in systems containing ethyl ether vapors. Stopcocks did not have to be regreased more frequently than when normal hydrocarbon greases are used with inert vapors.

Another possible base for this type of lubricant, which could be used in the presence of paraffin hydrocarbons, is triethanolamine. This material has a lower vapor pressure than glycerol (7×10^{-5} mm. at 25° C.) and the absorption of paraffin hydrocarbon vapors is about the same. In general, the same thickening agents can be used, but more care must be exercised to get the crystals in a sufficiently fine state of division.

Table I shows the comparative absorption of some organic vapors by anhydrous glycerol and triethanolamine, when a 2-gram sample with a surface area of 4.8 sq. cm. was exposed 26 hours at room temperature in a sealed jar containing the liquid organic solvent. The finished lubricants might be expected to absorb considerably less, since they are already saturated with

Table I. Absorption of Organic Vapors

Solvent	Glycerol	Triethanolamine
	Mg.	*Mg.*
Ethyl alcohol	300	330
Acetone	205	540
Ethyl ether	60	136
Carbon tetrachloride	17	55
Benzene	15	80
Petroleum ether (30–60)	5	5
	Glycerol Base	Apiezon M
Ethyl alcohol	200	2
Acetone	125	20
Ethyl ether	40	160
Carbon tetrachloride	9	940
Benzene	9	410
Petroleum ether (30–60)	2	300

the thickening agent and their viscosity is considerably increased.

The results of a comparison of the glycerol-base lubricant with a standard commercial stopcock grease, Apiezon *M*, are also shown in Table I. The vapors were absorbed here by a 1-gram sample; otherwise the experimental conditions were identical with those already described. The solvents used in making these experiments were chosen to cover a wide range of polarity and no attempt was made to collect compounds to which glycerol was resistant. The lubricant is useless with lower alcohols and ketones where excellent protection is afforded by the Apiezon M. It would probably be of value, however, with the higher members of these families where the solubilities in hydrocarbons become large.

The tests described were severe, since the area exposed to the vapor is many times that obtained with an ordinary stopcock or ground-glass joint. The effect of fifty liquid solvents on this type of lubricant has been recorded (*4*). The absorption results here are in good agreement.

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Determination of Phthalate

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NUMEROUS ternary systems involving phthalates have been investigated in this laboratory during the past 20 years. The analytical procedure for the determination of phthalate has necessarily been an indirect one, since no direct method known to the authors is satisfactory in aqueous solution.

Kappelmeier (*3*) determined phthalate in alkyd resins by the precipitation of potassium phthalate containing one molecule of alcohol of crystallization. This precipitation is carried out in benzene or in anhydrous alcohol-ether solution and is not applicable to aqueous solutions. Fonrobert and Muenchmeyer (*2*) determined phthalate in varnish and Thames (*4*) in plasticizers by precipitation of lead phthalate with lead acetate and conversion of this precipitate into lead sulfate which was weighed.

Zombory (*5*) found that lead could be determined gravimetrically by precipitation as lead phthalate in alcoholic solution. It was felt that possibly this procedure could be reversed and phthalate be determined by adding lead as the precipitating reagent. With this thought in mind, this investigation was undertaken.

Solubilities of lead phthalate in water and in various concentrations of alcohol were determined at various temperatures. It was found that at 25° C. the solubility of lead phthalate in 33% alcohol by volume is 2 mg. per 100 cc. of solution. The solubility does not decrease appreciably in higher concentrations of alcohol.

Both lead nitrate and lead acetate were independently used as precipitating agents, the former giving low results and the latter high results.

It is apparent that when lead nitrate is used as the precipitating agent, nitric acid is one of the by-products. The resulting solution is therefore acidic, accounting for the increased solubility of the lead phthalate which is a salt of a weak acid and consequently soluble in a strong acid.

An investigation was therefore made to find the optimum pH for precipitation. A solution made up of lead nitrate and excess sodium phthalate in 33% alcohol by volume as used by Zombory (*5*), showed a pH of 7.6 using the glass electrode. Determinations were then made using sodium phthalate and a calculated excess of lead nitrate in alcoholic solution of the same strength but with varying acid concentrations. From pH 2.8 to 6.4 low results were obtained. Above 6.4 high results were obtained.

This is explained by the solubility of lead phthalate in acid solution and the precipitation of lead hydroxide as the alkalinity increases. This latter fact was substantiated by determining that lead hydroxide starts to precipitate from alcoholic solutions when the pH reaches 5.1. Britton (*1*) found that lead hydroxide comes out of aqueous solution at a pH of about 6.

It is evident that the optimum pH value at which phthalate should be determined overlaps the pH value at which lead hydroxide forms. One cannot hope to make these two errors self-compensating, since excess lead nitrate will be present in varying amounts in determinations of phthalates in unknown solutions.

It seemed that by using lead acetate as the precipitating agent in place of lead nitrate, the by-product of the reaction would be the weak acetic acid and better results might be obtained. A new difficulty was encountered here, since the precipitate came down very finely divided and did not settle out upon standing, rendering filtration and washing virtually impossible.

A few determinations were completed by making the precipitation in aqueous solution and boiling the mixture before the addition of alcohol. After standing, the mixture was centrifuged and the precipitate washed and weighed. All the values obtained were high and the magnitude of the errors was not consistent. This is believed to be due to the contamination of the precipitate with varying amounts of basic lead acetate.

The evidence indicates that phthalate cannot be determined directly by the addition of lead nitrate or acetate to aqueous solutions containing phthalate ion.

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The material for this paper was taken from a thesis of John F. Strempfer presented to the Graduate Committee of Trinity College in partial fulfillment of the requirements for the master of science degree.

Stability of Wijs Solution for Iodine Number Determinations

FRANK A. NORRIS AND ROBERT J. BUSWELL, General Mills, Inc., Minneapolis, Minn.

THE Wijs solution is probably the most satisfactory for general use in determining iodine numbers. Its more widespread utilization is hindered mainly by its supposed difficulty of preparation and short life. The first objection is hardly valid if chlorine is available and if the analyst is reasonably careful. The short life would appear to be a much more serious objection, since three standard references on fat analytical methods specifically caution against using this solution when it is more than 30 days old (*1*, *2*, *4*). However, Hilditch (*3*) does not accept this view, and Wijs himself (*5*) claims indefinite stability for the reagent. Previous experience by one of the present authors is in agreement with the two last-named investigators.

Since a decision on the stability of Wijs solution was considered necessary in connection with some analytical work, the authors measured the stability of the reagent when stored at room temperature in 250-ml. dark bottles. The solution was prepared in the standard manner and contained 1.5% excess equivalents of iodine over chlorine. Linseed oil, stored in the ice box, was used as the test substrate.

Over a total period of 505 days, the Wijs solution did not change sufficiently to cause a measurable difference in the iodine number of the substrate. No measurable differences were found when the reagent was taken from bottles that had been previously opened. These facts indicate the validity of storing the solution a year or more, if storage is in small bottles which are opened as needed.

Table I. Stability of Wijs Solution

Bottle No.	Age of Solution, Days	Days Since Bottle First Opened	Iodine No.
1	6	0	177.3
2	45	0	178.0
2	97	52	177.1
3	97	0	...
3	132	35	176.5
3	174	77	176.8
3	231	134	176.3
4	277	0	177.0
4	314	37	177.4
4	374	97	177.0
5	404	0	177.8
5	455	51	176.8
5	505	101	177.2

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An Aid in Ashing Certain Materials

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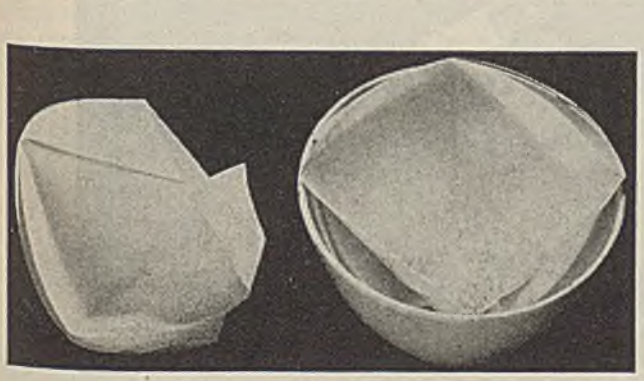
THE ashing of many products is often difficult and time-consuming, as is evidenced by the number of procedures published for special materials (*1*). This is especially true for substances that are finely ground, contain oil or fat, or have a high phosphorus-to-base ratio. For such materials, a simple procedure which was recently devised to ash large volumes of liquid egg white (*2*) is suggested.

This procedure consists of lining the crucible with filter paper, charring the material over a Meker burner in this lined crucible, moistening the char with a solution of magnesium chloride, and completing the ashing in a muffle furnace.

Filter paper of the correct size (Whatman No. 40 or equivalent) is folded as for filtering, the tip of the cone folded back, and this truncated cone fitted into the crucible. This liner prevents local overheating because of the air space between it and the crucible and permits the easy escape of the volatile materials,



since the charring proceeds slowly from the outside toward the center. The charred mass tends to retain its cone shape and is free of the crucible. Thus, the crucible walls are protected from the action of the materials ashed. Fatty materials burn more evenly and slowly, without spattering. The char is moistened with a solution of magnesium chloride added dropwise over the entire mass and ashed in a muffle overnight at 600° C. The ash obtained is light, fluffy, and voluminous and dissolves readily in dilute hydrochloric acid on heating.

If unaided by this procedure, materials which have a high phosphorus-to-base ratio fuse on ignition to a glassy mass which frequently entraps carbon that can then be burned off only with great difficulty. This fusion is caused by the conversion of the dihydrogen and monohydrogen phosphates upon ignition to the metaphosphate (*3*), which does not dissolve readily in hot dilute hydrochloric acid. In the suggested procedure, magnesium chloride supplies base to produce the tertiary phosphate which is unaffected on heating to 600° C.

In the authors' experience, samples ash poorly if they do not contain ample base for the phosphorus or in the case of plant material if the stalks, stems, hulls, coatings, or bran have been removed. In these materials, the use of the lined crucible and magnesium chloride is beneficial. In comparative trials, the proposed method aided the ashing of fresh tissues such as muscle, liver, intestines, and fat of chicken, pork, and beef as well as fresh egg white and yolk, casein, lecithin, corn gluten meal, flour, and starch.

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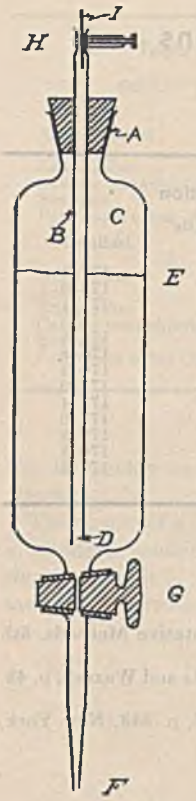

A Constant-Rate Dropping Funnel

GILBERT ASHBURN AND ROBERT L. FRANK,
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CHEMISTS are often annoyed to find, after carefully adjusting a dropping funnel to deliver a liquid dropwise into a reaction mixture, that within a short time the flow of drops has either stopped entirely or greatly diminished. A combination of two factors may be held largely responsible for this: the diminishing hydrostatic pressure as the liquid level falls, and the gradual tendency for the stopcock to close during the flow of the liquid. This conclusion can be readily verified by experiment.

A simple modification eliminates both these factors from an ordinary dropping funnel.

The modification, as shown in the accompanying diagram, chiefly consists in placing tightly in the top of the funnel a one-hole stopper, *A*, in which is inserted a glass tube, *B*, reaching nearly to the bottom of the funnel. When liquid is allowed to flow out, air enters tube *B* and escapes at *D*. The pressure in the air space, *C*, then changes in such a way that as the liquid level drops, the sum of *C* and the hydrostatic pressure represented by the distance *DE* remains essentially constant. This is in accordance with the principles of the Mariotte flask (*1*).

The gradual tendency of the stopcock to close may be obviated by restricting the entrance of air into *B* and then opening stopcock *G* completely. The air flow can be controlled either by means of a capillary tube attached to *B* or, as illustrated, *H*, by a screw clamp on a short piece of rubber tubing into which has been inserted a small wire, *I*. If the opening at *F* is of large diameter, it is sometimes necessary to draw out the tube in order to prevent air from entering at this point.

Dropping funnels equipped in this way have been found useful in this laboratory for the dropwise delivery of liquids into reaction mixtures. Although a slight decrease in rate can be noticed during the time of delivery, the flow is constant enough for most purposes.

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NEW EQUIPMENT

Sulfur Train

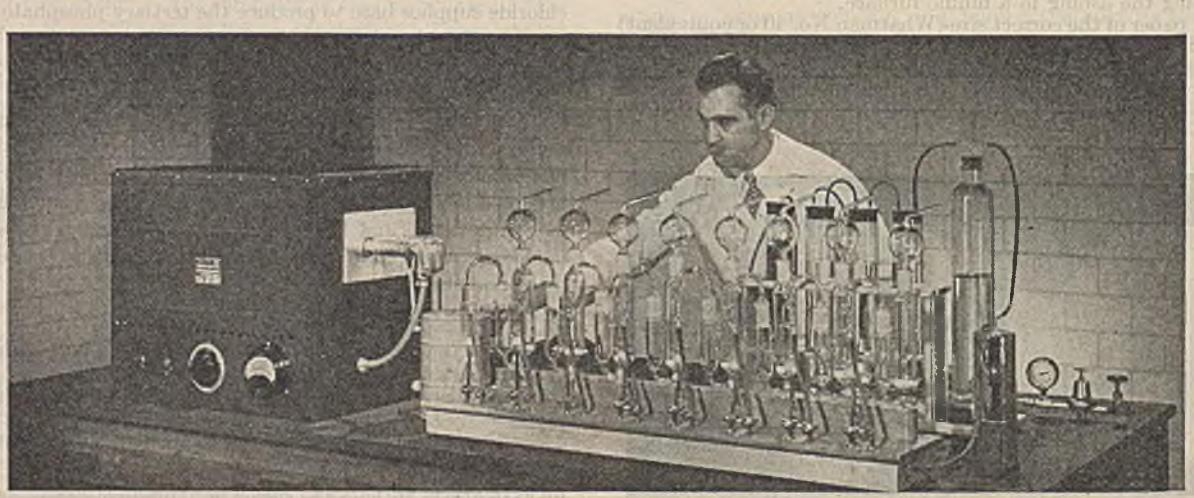
Numbered among improved apparatus accelerated by the war program is a sulfur train, originated by the Universal Oil Products Co. and built by the Precision Scientific Co., 1736 North Springfield Ave., Chicago. The method, identified as U.O.P. Method H-201-43-A, determines 0.0002 to 0.05% of sulfur in combustible liquids and gases. The samples are burned in a stream of purified air and the products of combustion absorbed in a solution of sodium hypobromite. The sulfur is then determined as sulfate by turbidimetric measurement of colloidal barium sulfate.

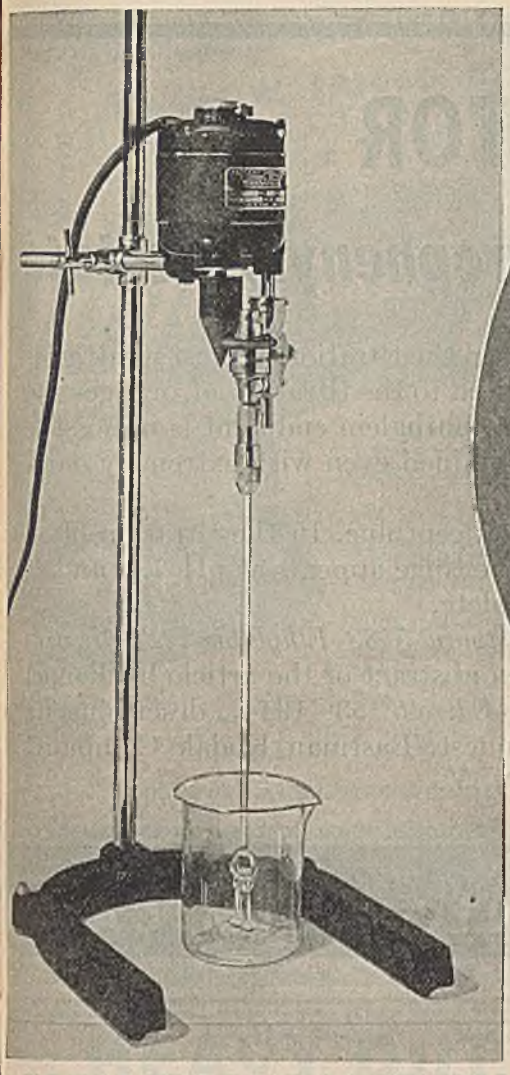
The apparatus consists of an air-pressure regulator and filter pressure gage, air-purification furnace, cooling tank, gas scrubbers, water regulator, moisture trap, and bank of eight A.S.T.M. sulfur lamps.

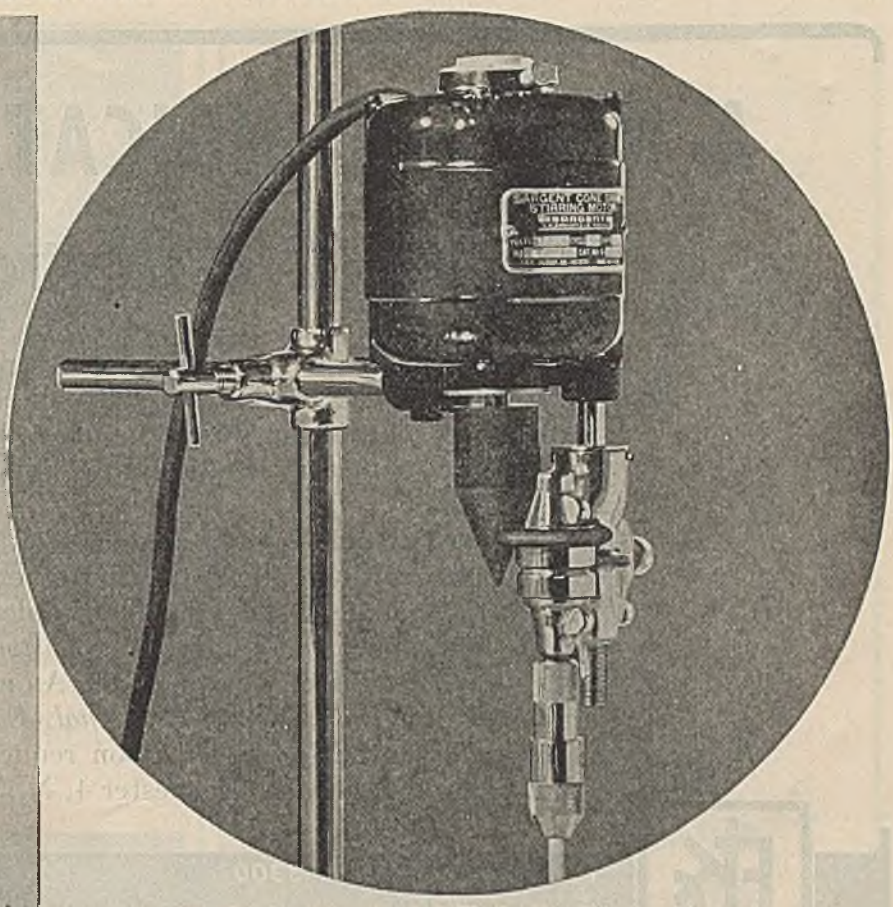
Compressed air controlled by a pressure-reducing valve is reduced to 2 p.s.i., then passes through an activated charcoal tower and into the air-purification furnace containing a heated stainless steel U-tube 1 inch in diameter and approximately 4 feet long, filled with quartz chips.

Air leaving the furnace is cooled to approximately room temperature by passing through a water-jacketed copper-coil condenser at the furnace outlet. It then passes successively through water, sodium hypobromite, and sodium hydroxide scrubbers, then through a constant-pressure regulator, into a moisture trap fitted into a vacuum bottle, and finally into a distribution manifold serving the sulfur lamps.

For the determination of sulfur in gases, the apparatus includes eight Duralumin weighing bombs of 35-ml. capacity, for connecting to the sulfur lamps.







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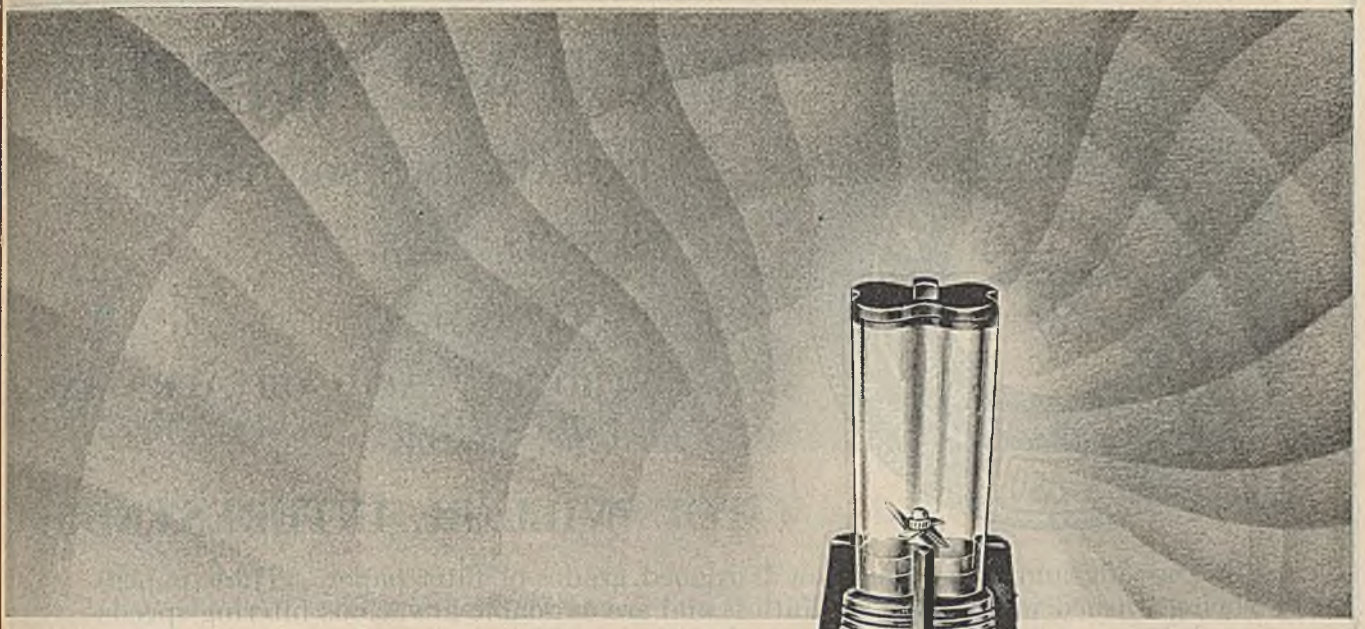

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