INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

WALTER J. MURPHY, EDITOR • ISSUED DECEMBER 22, 1943 • VOL. 15, NO. 12 • CONSECUTIVE NO. 24

28,600 copies of this issue printed.

Published by the American Chemical Society at Easton, Penna. Editorial Office: 1155 16th Street, N. W., Washington 6, D. C.; telephone, Republic 5301; cable, Jiechem (Washington). Business Office: American Chemical Society, 1155 16th Street, N. W., Washington 6, D. C. Advertising Office: 332 West 42nd Street, New York 18, N. Y.; telephone, Bryant 9-4430.

Entered as second-class matter at the Post Office at Easton, Penna., under the Act of March 3, 1879, as 24 times a year—Industrial Edition monthly on the 1st, Analytical Edition monthly on the 15th. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 13, 1918.

Remittances and orders for subscriptions and for single copies, notices of changes of address and new professional connections, and claims for missing numbers should be sent to the American Chemical Society, 1155 16th Street, N. W., Washington 6, D. C. Changes of address for the Industrial Edition must be received on or before the 18th of the preceding month and for the Analytical Edition not later than the 30th of the preceding month. Claims for missing numbers will not be allowed (1) if received more than 60 days from date of issue (owing to the hazards of wartime delivery, no claims can be honored from subscribers outside of North America), (2) if loss was due to failure of notice of change of address to be received before the dates specified in the preceding sentence, or (3) if the reason for claim is "missing from files".

Annual subscription—Industrial Edition and Analytical Edition sold only as a unit, members $3.00, nonmembers $4.00. Postage to countries not in the Pan-American Union $2.25; Canadian postage $0.75. Single copies—current issues, Industrial Edition $0.75, Analytical Edition $0.50; back numbers, Industrial Edition $0.80, Analytical Edition prices on request; special rates to members.

The American Chemical Society also publishes *Chemical and Engineering News*, *Chemical Abstracts*, and *Journal of the American Chemical Society*. Rates on request.

The Glass That Leaks

THROUGH TINY PORES

Speed, retentivity and freedom from chemical reaction—these are the outstanding characteristics of Pyrex brand Fritted Ware—the glass that filters through tiny pores. To meet varying filtration demands Corning Research has developed manufacturing methods providing uniformity in porosity.

Glass particles of uniform size, made from Pyrex brand Chemical Glass No. 774, are fritted into discs. These in turn are sealed into non-porous transparent glass bodies. No flux or glazing material is used.

"Pyrex" Fritted Ware is now available with five porosities of fritted discs. By judicious selection from this range, filtration of various sizes or types of precipitates can be accomplished at maximum speed.

FACTS ABOUT "PYREX" FRITTED WARE AND FILTERING APPARATUS

1. Fabricated from "Pyrex" brand Chemical Glass No. 774, *balanced* for mechanical strength, chemical stability and heat resistance.
2. Chemically stable—resistant to and insoluble in chemical reagents (except HF). Contamination kept to absolute minimum.
3. Complete visibility during filtration and extraction.
4. Not affected by filtration of solutions which destroy some other filter materials.
5. No loss of filtrates—large filtering area, non-porous sides.
6. Complete drying to constant weight at 110° C. May be heated in electric furnace to 150° C.
7. Discs may be subjected to pressures up to 15 lbs. psi.
8. Long life—readily cleaned with water or chemicals.

All items of "Pyrex" Fritted Ware are listed in Catalog LP21 and Supplements and are available through your laboratory supply dealer.

5 Porosities

"PYREX" FRITTED DISCS AND THEIR PRINCIPAL USES

EXTRA COARSE	Rapid Filtration Gas Dispersion and Filtration Extra support for other filter materials such as asbestos or glass fibre, sand or powdered glass
COARSE	Filtration of coarse precipitates Support for other filter materials Gas dispersion and filtration Mercury filtration Extraction
MEDIUM	Filtration of crystalline precipitates Gas dispersion and filtration Extraction
FINE	Filtration of fine precipitates such as $BaSO_4$
ULTRA FINE	Bacteria-free filtration

"YOU HAVE DONE A GOOD JOB OF SENDING GLASS TO WAR"

PYREX T.M. REG. U.S. PAT. OFF.

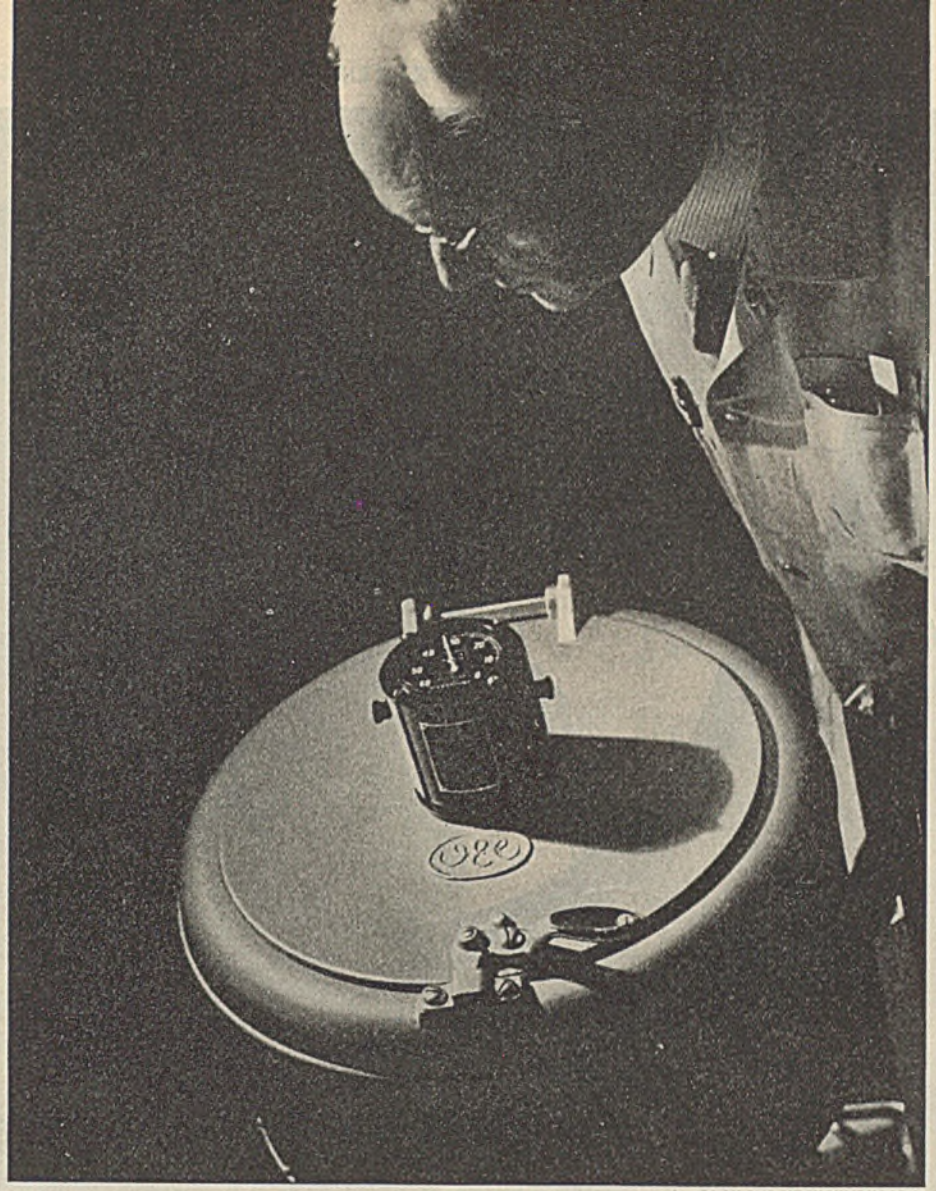
CORNING *means* Research in Glass

VYCOR TRADE MARK REG. U.S. PAT. OFF.

PYREX BRAND LABORATORY WARE

"PYREX" and "VYCOR" are registered trade-marks and indicate manufacture by

CORNING GLASS WORKS • CORNING, NEW YORK



Passed by a Master

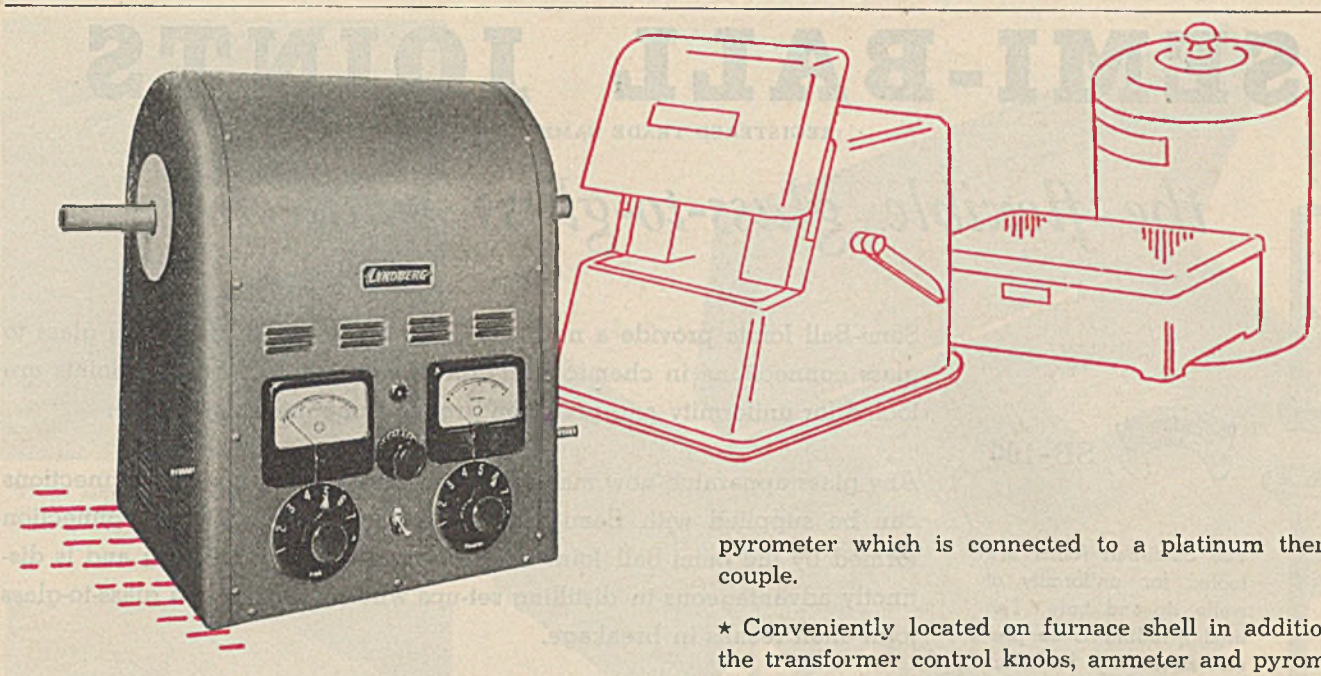
As American fighting ships leave the "ways" for service on the seven seas, International Centrifuges leave the factory testing floor for their own unique contribution to victory. They are accepted for service only on signal of approval from the factory superintendent, Mr. Smith. Forty years' experience with every kind of International Centrifuge has earned for him the title of "Master" and packs his signal of approval with significance. It means mechanical perfection in producing life-giving blood plasma for the use of our soldiers and sailors in the far-flung battle lines of the world. It is the consummation of numerous inspections of accurately machined parts to eradicate the possibility of flaw or imperfection. It is the final guarantee of complete coordination with all moving parts dynamically balanced. Like all other "Internationals" passed by a Master, these machines are ready for years of continuous and reliable operation.

Makers of Fine Centrifuges for More than Forty Years

INTERNATIONAL EQUIPMENT COMPANY

BOSTON, MASSACHUSETTS


A
Merrie
Christmas
and a
Happy
New Year
HELLIGE, INC.
3718 NORTHERN BOULEVARD · LONG ISLAND CITY 1, N. Y.



Dependability

IN YOUR LABORATORY!

THE NEW LINDBERG LABORATORY FURNACES AND HOT PLATES are engineered for dependable performance and built for long life. In addition, they are designed to add to the high standard of present day laboratory cleanliness. Even the most fastidious technician will appreciate the modern streamlined appearance Lindberg equipment presents.

Pictured above is the Lindberg Combustion Tube Furnace. Let's glance over the many important features you can benefit by in your laboratory.

★ Because of the high temperatures obtainable, this furnace is ideal for fast modern volumetric methods of carbon or sulphur determination as well as gravimetric determination on carbon and all alloy steels, including stainless and heat resisting steels.

★ Three low voltage, high temperature Globar elements permit continuous operation with temperatures up to 2500°F. or for occasional operation to 2650°F. Heating elements are easily accessible—no inconvenient dismantling and assembling job required to replace them. Temperature is regulated by a built-in variable voltage transformer with coarse or fine adjustment for amperage control. Adjustment is made by merely making the necessary settings with the amperage control knobs.

★ An ammeter is provided to show the proper and safe current required for the Globar heating elements. The temperature in the combustion zone is indicated by a pyrometer which is connected to a platinum thermocouple.

★ Conveniently located on furnace shell in addition to the transformer control knobs, ammeter and pyrometer are a pilot light which shows when furnace is in operation; an on and off switch; and adjusting knob for controlling oxygen flow through a needle valve and rubber tubing adapters connected to the needle valve.

★ Furnace is equipped with adapters for easy interchangeability of tubes. Three sizes of tubes, 1", 1¼", and 1½" O.D. can be adpated, while fourth tube, 2" O.D., can be used without adapters.

★ For 60 cycle, AC current with voltage at either 110 or 220 volts. Furnaces for 25 cycle operation can also be supplied.

See your dealer today. He will be pleased to go over your requirements and point out the advantages you get with the new Lindberg Combustion Tube Furnaces, Box Furnaces, Crucible Furnaces and the new laboratory Hot Plates.

LINDBERG

Well-known Throughout the World as the Leaders in Developing and Manufacturing Industrial Heat Treating Equipment

LINDBERG ENGINEERING COMPANY
2450 WEST HUBBARD STREET, CHICAGO 12, ILLINOIS

SOLD EXCLUSIVELY THROUGH LABORATORY EQUIPMENT DEALERS

SEMI-BALL JOINTS

(REGISTERED TRADE NAME)

the flexible glass-to-glass connection

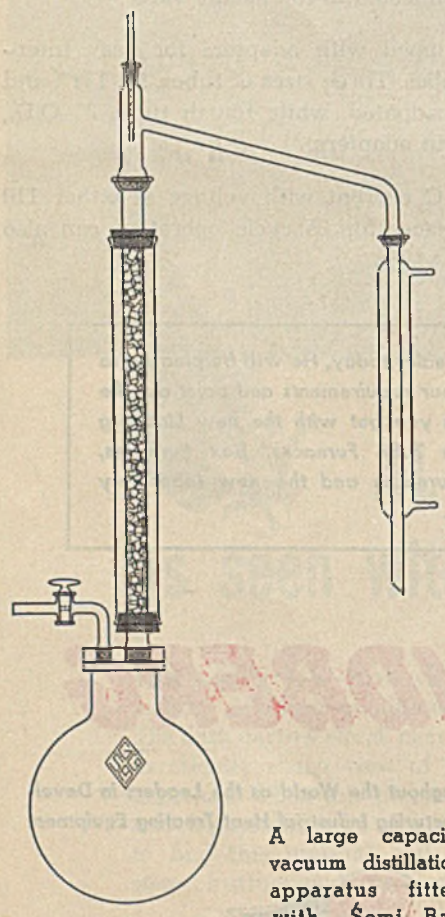
SB-100

The Semi-Ball Joints are tooled for uniformity of walls, lip and bore. The final grinding is so precise as to allow for interchangeability of parts.

Semi-Ball Joints provide a new and convenient means of making glass to glass connections in chemical laboratory apparatus. Semi-Ball Joints are tooled for uniformity and precision ground to be interchangeable.

Any glass apparatus now made with our famous "Inter-Joint" connections can be supplied with Semi-Ball Joints where needed. The connection formed by the Semi-Ball Joint is free from sticking or freezing and is distinctly advantageous in distilling set-ups where freezing of a glass-to-glass joint often results in breakage.

The size numbers assigned to Semi-Ball Joints designate the two essential dimensions in millimeters. In the numerator, the number refers to the diameter of the ball and in the denominator the number refers to the diameter of the bore through the connection.



A large capacity vacuum distillation apparatus fitted with Semi-Ball Joints. Note that the use of Semi-Ball Joints in an installation of this type prevents breakage from strains due to improper mounting of apparatus.

SEMI-BALL CLAMPS

Made of cadmium plated steel.

CLAMP SIZE	FOR SEMI-BALL SIZE	PRICE EACH
12	12/1 to 12/5	.50
18	18/7 to 18/9	.50
28	28/11 to 28/15	.50
35	35/20 to 35/25	.50
40	40/25	.75
50	50/30	.75
65	65/40	1.00
75	75/50	1.75
102	102/75	5.00

SB-310

SB-360

SB-410

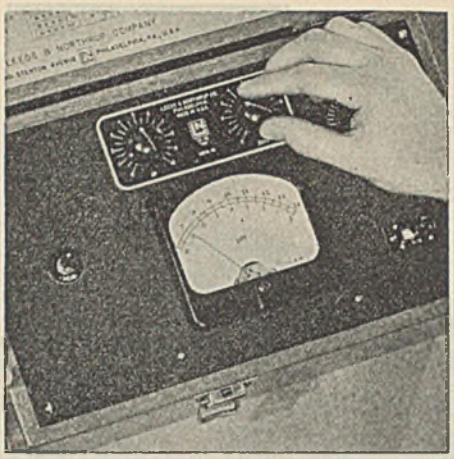
SB-200

SB-210

A 16 page bulletin illustrating many standard items equipped with Semi-Ball Joints is now available.

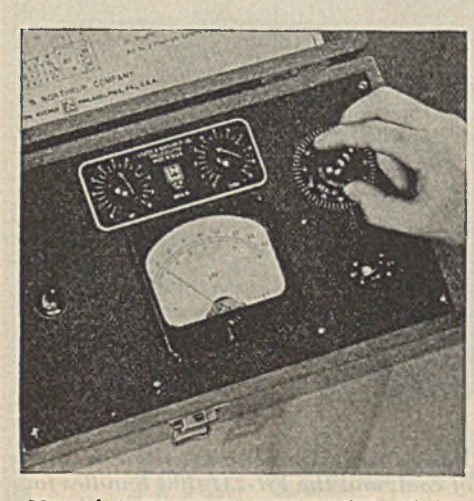
Write for your copy today.

SCIENTIFIC GLASS APPARATUS COMPANY
BLOOMFIELD, N.J.

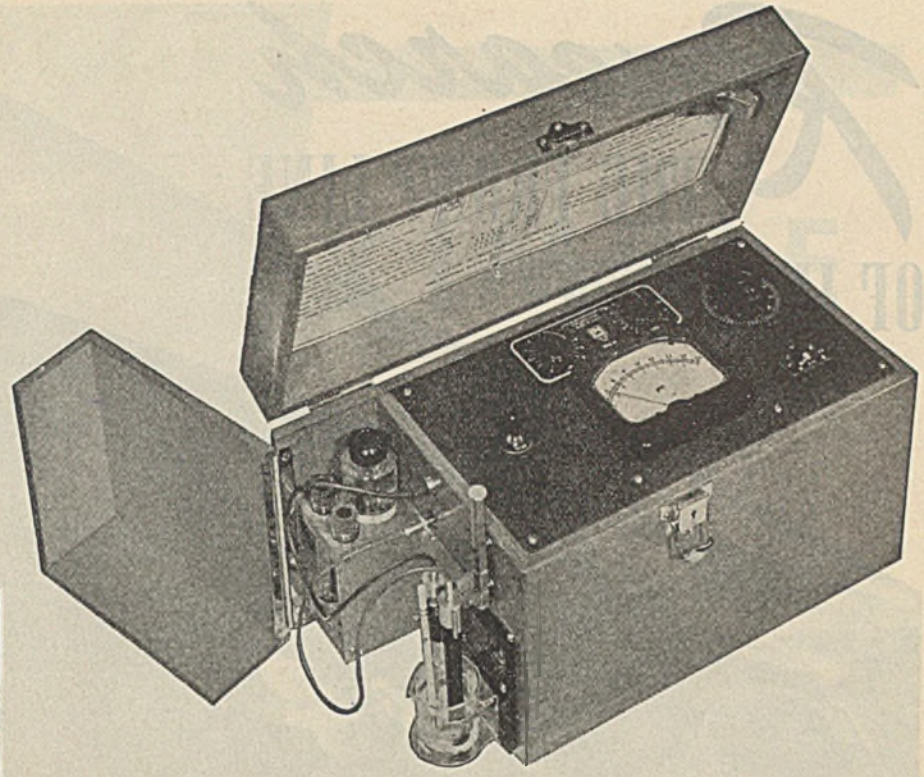


Three simple adjustments make the instrument ready for measurements. First, instrument zero is adjusted with a single knob.

Electrode calibration is compensated by a second knob. Only the temperature compensator need be reset for later tests.



Manual temperature compensator, set to solution temperature, eliminates computations, thus saving time and avoiding errors.



MEASURING pH WITH GLASS ELECTRODES?

Here, at $160, is our Indicator which Sets New Standards of Speed and Convenience

Although simplified and stripped to essentials for low cost and easy, rapid operation, L&N's Glass-Electrode pH Indicator has a limit of error of only ±0.1 pH. Measurements are made by simply pouring the sample into the beaker and reading pH directly.

Outstanding advantages include:

1. Full accuracy in atmospheres of 95% relative humidity up to 30 C.
2. Manual temperature compensator eliminates computations.
3. Instrument is adequately shielded from electrical disturbances.
4. The double range, covering 0–8 and 6–14 pH, provides a convenient overlap of 2 pH...a desirable feature for titrations.
5. The calibrated scale is longer than in any comparable pH indicator.
6. The mahogany case stands severe use; is not affected by high humidity.
7. Dry cells have their own compartment; can't corrode wiring, etc.
8. A whole end of the box swings out, leaving the electrodes and sample holder easily accessible.
9. Factory filled and sealed electrodes are highly stable.
10. Sample cup is a 50 ml beaker.
11. Fifteen-inch shielded leads permit electrodes to be used outside the case, for titration as well as pH.
12. Only 3 simple preliminary adjustments are necessary. Each is independent of the other.
13. Maintenance is negligible, consisting of adding KCl to the reference-electrode salt bridge every 6 or 8 weeks, cleaning the electrodes occasionally, and replacing batteries at infrequent intervals.

This Indicator is moderately priced for quality at $160.00. It comes complete with everything necessary for pH measurements.

Full details in Catalog E-96(2).

UNIFORM *as the fighting 28th*

WHEN sturdy young Pennsylvanians of the Keystone Division parade past, blood-red insignia proudly worn on husky shoulders stamps them as members of the famed 28th — in this war as in the last among this earth's toughest fighting men.

As America is depending upon young huskies of the 28th, so American chemists may depend upon ANALYTICAL REAGENTS in containers stamped with the name of MALLINCKRODT. MALLINCKRODT'S ANALYTICAL REAGENT label is known as a symbol of high standards for accurate gravimetric, colorimetric, gasometric or titrimetric work. Send for a catalogue of MALLINCKRODT ANALYTICAL REAGENTS and other chemicals for laboratory use.

ALWAYS SPECIFY REAGENTS IN MANUFACTURER'S ORIGINAL PACKAGES

FINE CHEMICALS
Mallinckrodt
SINCE 1867

MALLINCKRODT CHEMICAL WORKS

76 years of service to chemical users

ST. LOUIS • PHILADELPHIA • MONTREAL
CHICAGO • NEW YORK • LOS ANGELES

INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

PUBLISHED BY THE AMERICAN CHEMICAL SOCIETY • WALTER J. MURPHY, EDITOR

Polymer Distribution of Varnish Resins

H. E. ADAMS AND P. O. POWERS, Armstrong Cork Company, Lancaster, Penna.

An approximate estimate of the distribution of polymers in varnish resins may be made by precipitating the resin from dilute solution with measured amounts of a nonsolvent, while determining the decrease in light transmission through the medium. This titration is reasonably rapid and can be carried out in 20 minutes. This method has been used with hydrocarbon resins, rosin-modified phenolic and maleic resins, and pure phenolic resins using toluene as the solvent and methanol or hexane as the nonsolvent. The titration curves obtained in this manner may be used to compare resins of the same type. To estimate polymer distribution from these curves, the concentration of nonsolvent at which carefully separated fractions of a resin are precipitated must be determined.

THE distribution of polymers in commercial resins used in the manufacture of paints and varnishes has never been reported. Since these resins are considerably lower in molecular weight than the resins used as plastics whose polymer distribution has in many cases been established, the determination might be expected to be rather simpler. However, the polymers in commercial resins are often built up from several different types of molecules. This results in a greater complexity in the types of polymers formed.

Determination of the cloud point (*2*) of varnish resins in many instances gives a useful index of the solubility and hence the degree of polymerization of a resin. It has been found in some cases, however, that two resins of the same type and of the same cloud point are not identical in all their properties. This difference is probably due to a difference in the distribution of the polymers in the two resins. It was desired to develop a method that would quickly establish such differences.

Polymer distribution has often been effected by fractional precipitation of the resin from solution. The fractions are separated and their properties determined. This method is time-consuming and while it has been used in these laboratories, it is much too slow for routine examination of samples of resins.

Nephelometric methods have been used widely to measure the amount of a precipitate. McNally (*1*) has described a method for measuring the transmission of light through a solution of a synthetic resin during precipitation by a nonsolvent. A photronic cell was used to measure the transmission, which varied inversely with the amount of resin precipitated.

Schulz (*3*) has shown that the amount of nonsolvent required to precipitate a polymer is a measure of its degree of polymerization. Thus the distribution of polymers in a resin may be estimated if the transmission during precipitation and the concentration of nonsolvent required to precipitate a polymer are known. Even where the polymer-nonsolvent-concentration relationship has not been established, the results of precipitation are valuable in comparing resins of the same class.

Apparatus and Method

The titration was conducted in a square pint jar which had been painted black except for two windows on opposite sides about 2.5 × 7.5 cm. (1 × 3 inches). Light entered through one window from an automobile head lamp with a parabolic reflector, using a 6-volt storage battery as a source of current. The reflector was covered except for a small window, placed in front of the titration jar (Figure 1). The photronic cell (Weston No. 594) was placed in front of the other window. The circuit used to measure the cell voltage is shown in Figure 2.

A 1.5-volt dry cell was used as source of voltage. The poten-

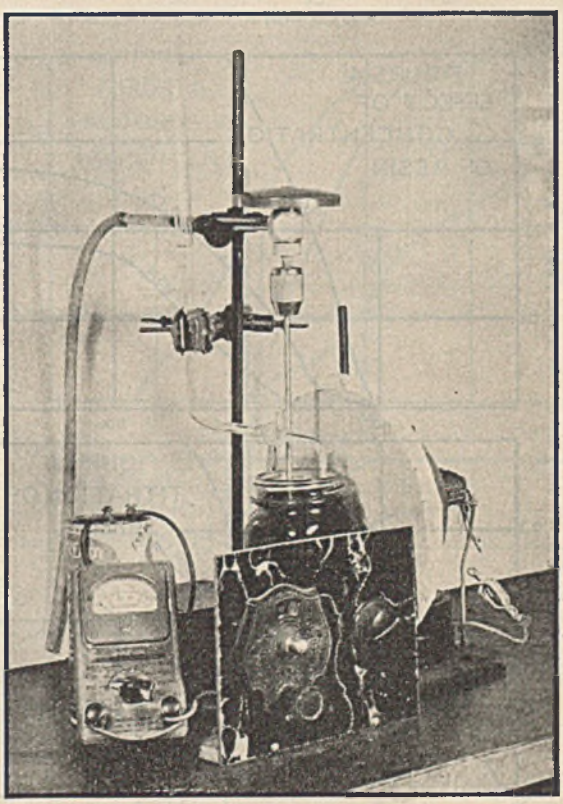


FIGURE 1. TITRATION APPARATUS

tiometer was wire-wound and had a spread of about 180°. A dial reading 0 to 100 was used to measure the position of the null point. Resistances R_1 to R_5 were carbon resistors. The values are those actually used, but are not critical. These resistors make it possible to titrate solutions of dark-colored resins, since changes in transmission at low intensities can be measured if a high resistance is used.

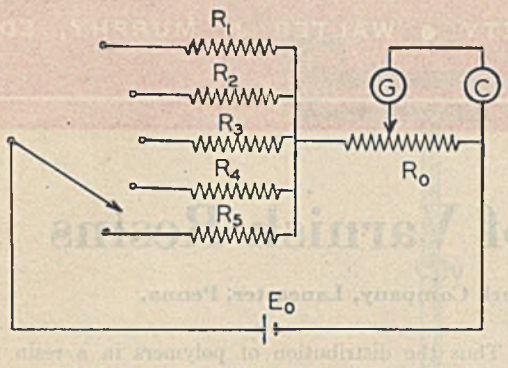


FIGURE 2. PHOTRONIC CELL CIRCUIT

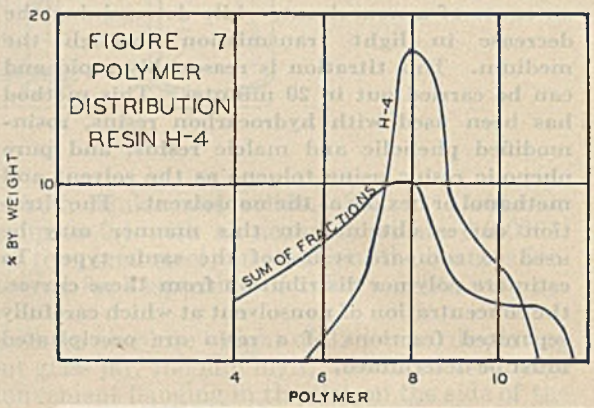
R_0.	1092 ohms	E_0.	1.5 volts
R_1.	057 ohms	C.	Photronic cell
R_2.	5108 ohms	G.	Microammeter
R_3.	9489 ohms		
R_4.	27,500 ohms		
R_5.	97,000 ohms		

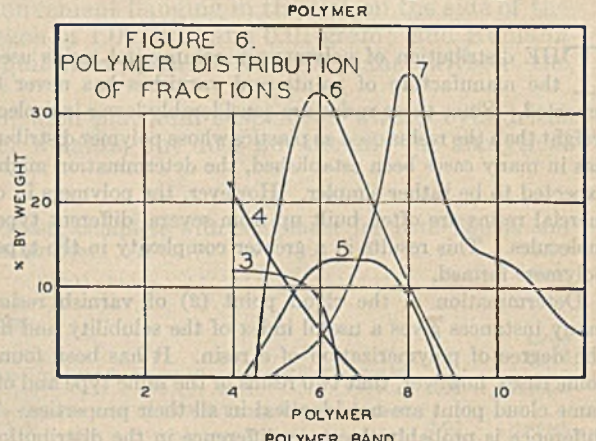
The solution of the resin and the nonsolvent were brought to 25° C. To conduct a titration, 80 cc. of the solution of the resin, usually in toluene at 0.05 per cent solids, were added to the jar and the nonsolvent was added with stirring by an air-driven agitator. The rate of stirring must be sufficiently great to ensure complete mixing, but must not cause the introduction of air bubbles into the solution. A nonsolvent, usually methanol, was added until it was apparent that precipitation was about to occur. The microammeter was balanced, using the switch setting giving the largest reading on the potentiometer scale. Small amounts of methanol were added and the circuit was again balanced. The reading just before precipitation started was taken as a measure of the initial transmission, I_0. Small amounts of precipitant were added and the transmission, I, was determined after each addition. Decrease in transmission occurs rapidly at first and the titration is complete when further addition of precipitant does not further decrease the transmission.

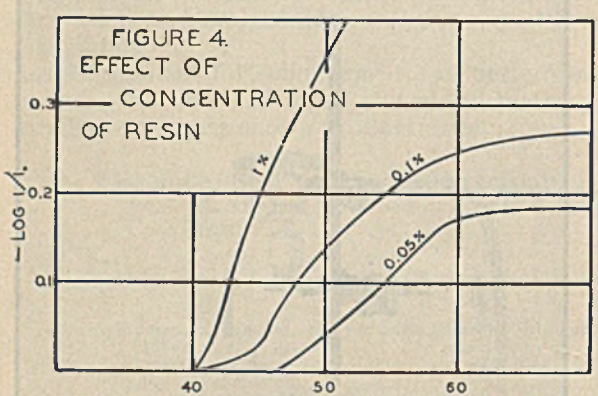
It might be expected that transmission would increase by dilution, but generally such increase was not observed. The apparatus and method were checked by precipitating inorganic salts and it was found the extinction, $-\log I/I_0$, measured the amount of the precipitate at low concentration.

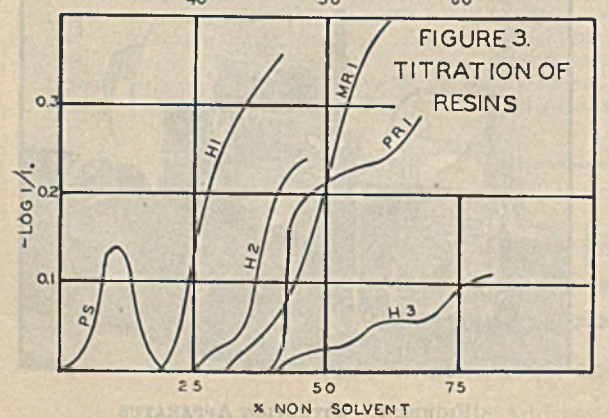
Titration of Varnish Resins

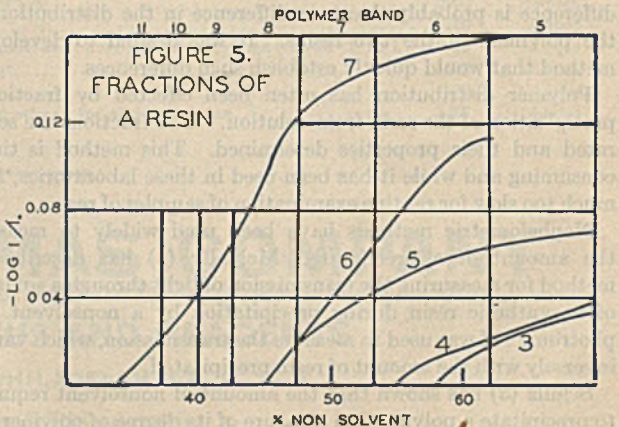
To establish the range of precipitation and conditions for titration, a variety of varnish resins was dissolved in toluene (0.5 per cent solution) and precipitated by the addition of methanol. Figure 3 shows that an estimate of polymer distribution is possible for several types of varnish resins. H1, H2, and H3 are coumarone-indene resins of successively decreasing molecular weight. A maleic rosin resin (MR1) and a rosin-modified phenolic (PR1) resin were also titrated, and the results are shown in Figure 3. These resins are typical of the less soluble varnish resins. The











results show the wide range of composition of commercial resins. Polystyrene (PS) is also included; it is much higher in molecular weight than the other resins. The decrease in transmission is due to the resin settling out of solution; this can be overcome by using a more dilute solution.

Titrations are probably more significant if run at lower concentration. Figure 4 shows the results of titrating the same resin at three different concentrations. It will be noted that higher concentration of nonsolvent may be required to start precipitation at low concentration. Estimates of molecular size from precipitability determinations may therefore depend on concentration of the resin, which should be constant for such determinations.

Polymer Distribution

The titration curves are believed to be a sufficient basis for comparison of commercial varnish resins. However, to correlate a titration with polymer distribution, a coumarone-indene resin was fractionated by partial solution, resulting in seven fractions, and the molecular weight of the fractions was determined by depression of the melting point of pure benzene.

The titration curves of these fractions are shown in Figure 5. The first two more soluble fractions did not precipitate on titration. The other fractions gave varying amounts of precipitate, only the last fraction being entirely precipitated, showing presence of lower polymers in these fractions.

Since the results of the titration and molecular weight determinations indicated that the decamer was precipitated at 37 per cent nonsolvent and the tetramer at 73 per cent nonsolvent, the Schulz (*3*) equation

$$\gamma^* = A + B/X$$

where

γ^* = concentration of nonsolvent
X = degree of polymerization

and

A and B = constants

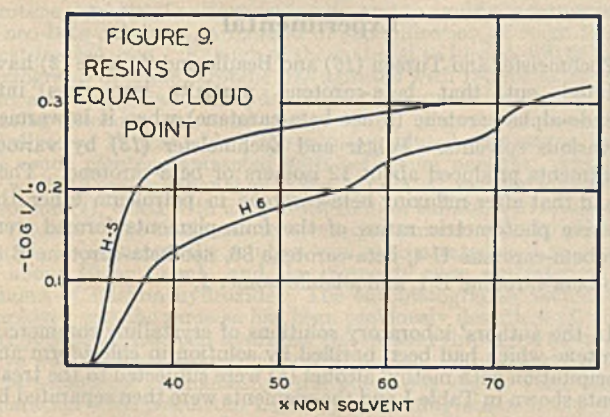
was used to estimate the range in which each polymer is thrown down. From the above values

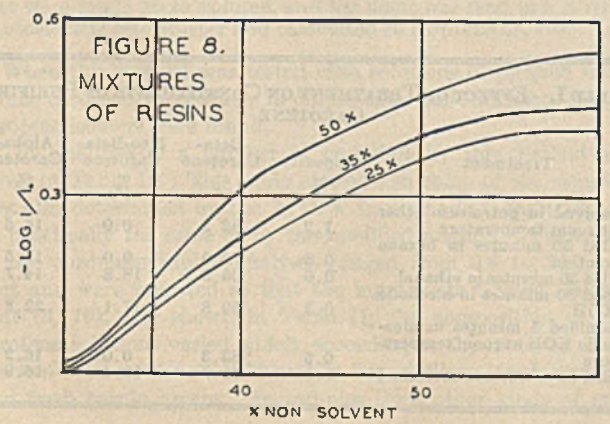
$$\gamma^* = 0.13 + \frac{2.42}{X}$$

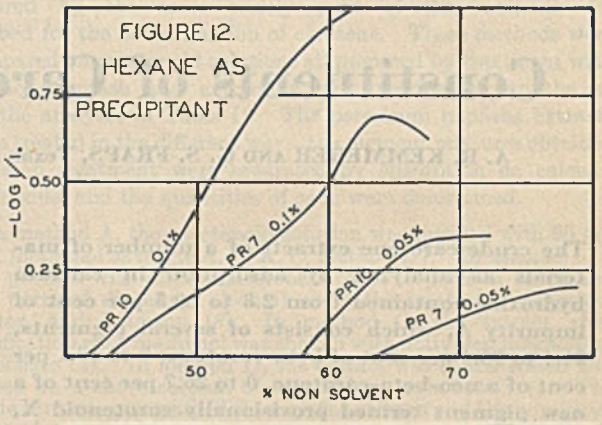
The range for each polymer by this relationship is shown in Figure 6. The amount in each fraction is estimated by the differential of $-\log I/I_0$ and the values for the fractions are summed in Figure 7. A similar estimate of the distribution of the resin was made from the titration of the original resin and, as might be expected, shows a somewhat narrower distribution. It is apparent that the lower polymers are not estimated by this method. The results also suggest a mutual solubility effect of various polymers, resulting in incomplete separation.

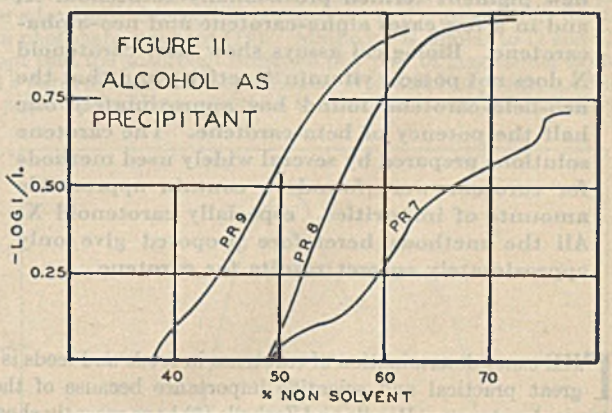
In many cases the high polymers determine the character of the resin, and an estimate of the range and amount of these polymers afford significant information on the composition of a resin.

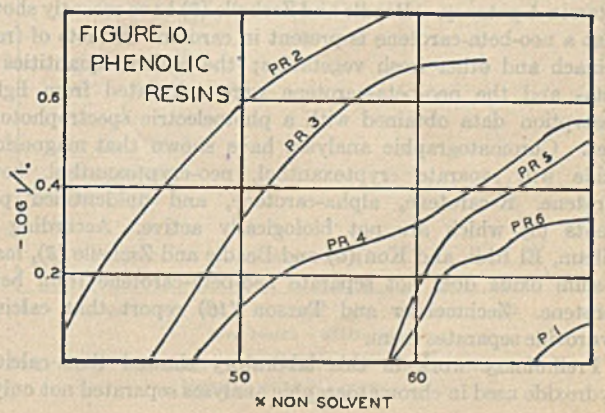
To determine if titration would measure small differences in the composition of a resin, mixtures of two hydrocarbon resins of somewhat similar properties were examined. It will be noted (Figure 8) that the curves indicate the presence of larger amounts of higher polymers as the content of the less soluble resin is in-











creased. Resins of equal cloud point may have somewhat different properties. The titration curve of two such resins, H5 and H6, is shown in Figure 9. Resin H5 has a higher content of large polymers.

Titration of Rosin-Modified Phenolic Resins

Titration curves were determined for samples of several commercial rosin-modified phenolic resins, PR2–6, and of a "pure" phenolic resin, P1 (Figure 10). The resins having the greatest amount of material precipitated, as measured by $-\log I/I_0$, at low nonsolvent concentrations are the more viscous, higher melting, and less soluble. The pure phenolic, P1, is one of the more soluble of its class.

No attempt has been made to estimate polymer distribution from these titrations, as data are not available on molecular weight and precipitability of fractions of these resins. However, it is known that ester gum is not precipitated under the conditions of titration. Thus the material precipitated is phenolic resin and condensates of the phenolic resin with rosin glyceride.

A series of rosin-modified phenolic resins, PR7, 8, 9, and 10, was titrated. These resins differ chiefly in the content of phenolic resin which increases in each successive sample. The precipitation with methanol is in agreement with this composition (Figure 11). However, the results with PR10 were unexpected, since its titration curve nearly coincided with that of PR7. It is believed that the higher content of polar groups introduced by the higher phenolic content is responsible for this result. Titrating PR7 and PR10 with hexane the resins are in the expected order. The results at two concentrations are shown in Figure 12.

Conclusions

A rapid and simple method for the estimation of polymer distribution by titration of a solution of a varnish resin with nonsolvent has been used with hydrocarbon and modified phenolic resins, and minor differences in resins can be quickly shown. The method outlined here is tentative and better conditions of determination are undoubtedly possible.

Literature Cited

(1) McNally, J. G., Gibson Island A. A. A. S. Conference (1942).
(2) Powers, P. O., Ind. Eng. Chem., Anal. Ed., 14, 387 (1942).
(3) Schulz, G. V., *Z. Phys. Chem.*, A179, 321 (1937).

Presented before the Division of Paint, Varnish, and Plastics Chemistry at the 106th Meeting of the American Chemical Society, Pittsburgh, Penna.

Constituents of Carotene Extracts of Plants

A. R. KEMMERER AND G. S. FRAPS, Texas Agricultural Experiment Station, College Station, Texas

The crude carotene extracts of a number of materials as analyzed by adsorption in calcium hydroxide contained from 2.3 to 39.5 per cent of impurity A, which consists of several pigments, 26.4 to 95.4 per cent of beta-carotene, 0 to 18.1 per cent of a neo-beta-carotene, 0 to 26.7 per cent of a new pigment termed provisionally carotenoid X, and in a few cases alpha-carotene and neo-alpha-carotene. Biological assays show that carotenoid X does not possess vitamin A activity and that the neo-beta-carotene found has approximately one half the potency of beta-carotene. The carotene solutions prepared by several widely used methods for carotene were found to contain appreciable amounts of impurities, especially carotenoid X. All the methods heretofore proposed give only approximately correct results for carotene.

THE exact determination of carotenes in foods and feeds is of great practical and scientific importance because of their vitamin A potency. Beadle and Zscheile (*2*) have recently shown that a neo-beta-carotene is present in carotene extracts of fresh spinach and other fresh vegetables; the relative quantities of beta- and the neo-beta-carotene were calculated from light-absorption data obtained with a photoelectric spectrophotometer. Chromatographic analyses have shown that magnesium oxide will separate cryptoxanthol, neo-cryptoxanthol, beta-carotene, K-carotene, alpha-carotene, and unidentified pigments (*3*) which are not biologically active. According to Gillam, El Ridi, and Kon (*6*) and Beadle and Zscheile (*2*), magnesium oxide does not separate neo-beta-carotene from beta-carotene. Zechmeister and Tuzson (*16*) report that calcium hydroxide separates them.

Preliminary work in this laboratory showed that calcium hydroxide used in chromatographic analyses separated not only a neo-beta-carotene from beta-carotene, but also a carotenoid which has not previously been reported to occur naturally, here termed carotenoid X. Further study of the constituents of crude carotene obtained by different methods and from various sources is thus necessary.

Experimental

Zechmeister and Tuzson (*16*) and Beadle and Zscheile (*2*) have pointed out that beta-carotene partially isomerizes into pseudo-alpha-carotene (a neo-beta-carotene) when it is warmed in various solvents. Polgár and Zechmeister (*13*) by various treatments produced about 12 isomers of beta-carotene. They found that after refluxing beta-carotene in petroleum ether the relative photometric ratios of the four pigments formed were neo-beta-carotene U 4, beta-carotene 86, neo-beta-carotene B 8, neo-beta-carotene E 1, and a labile isomer 1.

In the authors' laboratory solutions of crystalline commercial carotene which had been purified by solution in chloroform and precipitation with methyl alcohol (*6*) were subjected to the treatments shown in Table I and the pigments were then separated by

Table I. Effect of Treatment on Constituents of Purified Carotene

Treatment	Impurity %	Beta-Carotene %	Neo-Beta-Carotene %	Alpha-Carotene %
Dissolved in petroleum ether at room temperature	1.2	82.2	0.0	16.6
Boiled 30 minutes in hexane solution	0.6	72.9	9.0	17.5
Boiled 30 minutes in ethanol	0.6	65.9	18.8	14.7
Boiled 30 minutes in alcoholic KOH	0.3	59.8	17.1	22.8
Saponified 5 minutes in alcoholic KOH at room temperature	0.5	83.3	0.0	16.2
Boiled 30 minutes in methanol	1.0	71.7	10.4	16.9

TABLE II. CONSTITUENTS OF CRUDE CAROTENE

	Crude Carotene, A. O. A. C. Method	Crude Carotene, Cold Saponification	Loss in $Ca(OH)_2$ Column	Constituents				
				Impurity A	Carotenoid X	Beta-carotene	Neo-beta-carotene	Alpha-carotene
	P. p. m.	*P. p. m.*	%	%	%	%	%	%
Alfalfa, dehydrated	25.0	23.5	3.7	27.0	0.0	66.4	6.6	...
	200.0	172.5	4.8	9.4	12.6	65.9	10.1	2.0
	...	31.7	7.9	30.8	26.1	37.5	5.6	...
	...	105.0	10.5	14.9	20.2	46.8	18.1	...
	...	142.0	8.8	12.7	20.5	50.2	16.6	...
	...	102.5	2.8	29.7	26.7	26.4	17.2	...
	...	120.0	8.7	10.7	0.0	75.3	14.0	...
	...	188.3	0.4	5.8	23.1	53.3	17.8	...
Apricots, canned	12.8	11.4	3.1	30.8	5.9	56.1	7.2	...
Bermuda grass, fresh	56.4	54.0	7.4	6.4	8.4	80.0	5.2	...
Bur clover, fresh	...	80.0	12.5	3.7	0.0	89.1	7.2	...
Carrots, fresh	36.9	39.3	2.5	6.5	0.0	64.7	0.0	28.8
Carrots, dehydrated	...	648.0	7.4	5.7	0.0	63.3	2.3	28.7
	...	1048.0	6.4	2.8	1.2	66.4	3.3	26.3
Collards, fresh	38.8	42.0	6.5	5.7	8.0	79.0	7.3	...
Hegari silage	4.8	5.1	20.3	31.1	16.0	52.9	0.0	...
Johnson grass, fresh	144.8	138.0	11.7	6.7	12.4	73.1	6.5	1.3
Oat plants, fresh	52.0	53.6	0.4	6.3	9.4	78.0	6.3	...
Sumac sorghum silage	...	4.0	10.0	39.5	18.8	37.5	4.2	...
	10.8	10.0	9.0	32.5	14.5	45.6	7.4	...
Sweet potatoes, fresh	45.0	43.0	2.8	11.7	5.4	77.8	3.7	1.4
Swiss chard, fresh	59.8	59.8	1.5	3.6	0.0	83.5	11.2	1.7
Turnip greens, fresh	42.8	41.3	8.5	4.6	0.0	95.4	0.0	0.0

chromatographic analysis in a column of calcium hydroxide. The amounts of each pigment were determined in terms of carotene by means of a KWSZ photoelectric colorimeter (*9*).

The purified carotene and the purified carotene treated with alcoholic potash at room temperature did not contain neo-beta-carotene. When the carotene was boiled in a hexane solution for 30 minutes, the resulting pigments contained 9.0 per cent of a neo-beta-carotene, probably neo-beta-carotene B (*13*); when boiled in methanol, 10.4 per cent; when boiled with alcoholic potassium hydroxide, 17.1 per cent; and when boiled with ethanol, 18.8 per cent. This shows that methods which require heat cannot be used in extracting carotene from plants when neo-beta-carotene and beta-carotene is to be determined.

In order to ascertain the percentages of the constituents in the carotene extracts of various materials, and to avoid the formation of neo-beta-carotenes, a method of saponification at room temperature was used. Enough of the material to furnish 100 to 200 micrograms of crude carotene was agitated with 150 ml. of 12 per cent alcoholic potassium hydroxide for 5 minutes in the jar of a Waring Blendor at room temperature. Petroleum naphtha (Skellysolve F) was added, the solution diluted with water, and the crude carotene extracted with petroleum naphtha, purified by washing with methanol as in the A. O. A. C. method for crude carotene (*1*), dried with anhydrous sodium sulfate, and made up to volume. The crude carotene was determined with the photoelectric colorimeter, the solution was concentrated in a vacuum to about 10 or 15 ml., and the pigments were separated in a column of calcium hydroxide. The chromatographic technique employed was the same as has been previously described (*3*, *10*), except that commercial hydrated lime passed through a 65-mesh sieve was used instead of magnesium oxide. The bands of color were separated mechanically, the color was eluted from each separation with petroleum naphtha containing ethanol, the eluates were made up to volume, and the color was read in a KWSZ photoelectric colorimeter and calculated to carotene (*3*, *10*).

When this method was tested with solutions of purified crystalline carotene, or carotene in oil, no detectable amounts of any carotene isomers were found.

The analyses of a number of materials by this method are given in Table II. The crude carotene in some of the samples was also determined by the A. O. A. C. method (*1*) and found to be practically the same as by the modified method. The losses in the chromatographic analyses ranged from 0.4 to 20.3 per cent and were prorated so that the ingredients totaled 100 per cent (*3*, *10*). As shown in Table II, the composition of the carotene solutions varied widely according to the material used. No neo-beta-carotene was found in hegari silage, fresh carrots, and fresh turnip greens. In samples from other kinds of materials the quantities ranged from 2.3 to 18.1 per cent. Carrots contained 26 to 29 per cent of alpha-carotene, and very small amounts were found in some of the other samples. Impurity A, which includes several bands in the upper part of the column above carotenoid X, ranged from 2.8 per cent of the pigments with carrots to 39.5 per cent with sumac sorghum silage. Alfalfa meal contained from 5.8 to 30.8 per cent. Apricots contained 30.8 per cent of impurity A, a portion of which was lycopene. Carotenoid X, which formed a band just above the beta-carotene, ranged from 0 to 26.7 per cent. The beta-carotene was as low as 26.4 per cent in one sample of alfalfa and as high as 95.4 per cent in fresh turnip greens. The neo-beta-carotene band was below the beta-carotene and above the alpha-carotene band, when alpha-carotene was present.

It is desirable to know the quantity of the various constituents of carotene secured by the more widely used of the methods described for the determination of carotene. These methods were compared on carotenoid solutions all prepared by treatment with alcoholic potash and extraction with petroleum naphtha as for the analyses in Table II. The petroleum naphtha extracts were treated in the different ways, the pigment mixtures obtained by each treatment were separated by adsorption on calcium hydroxide, and the quantities of each were determined.

In method A, the carotenoid solution was purified with 90 per cent methanol as in the A. O. A. C. method (*1*). In method B, it was purified with dilute diacetone alcohol (6 parts of water and 100 parts of diacetone alcohol) as in the method of Hegsted, Porter, and Peterson (*3*). In method C, the extract after purification with methanol was shaken with activated magnesium carbonate (*4*). In method D, the extract was concentrated and placed on a short column of magnesium carbonate (*11*). The crude carotene was washed through the column and the impurities were adsorbed. Method E is the same as D, except that dicalcium phosphate was used (*12*). Method F is similar to D and E, except that the adsorbent consisted of 1 part of magnesium oxide and 3 parts of Supercel as specified by Wall and Kelly (*14*). After the pigments had been adsorbed, the crude carotene was eluted by

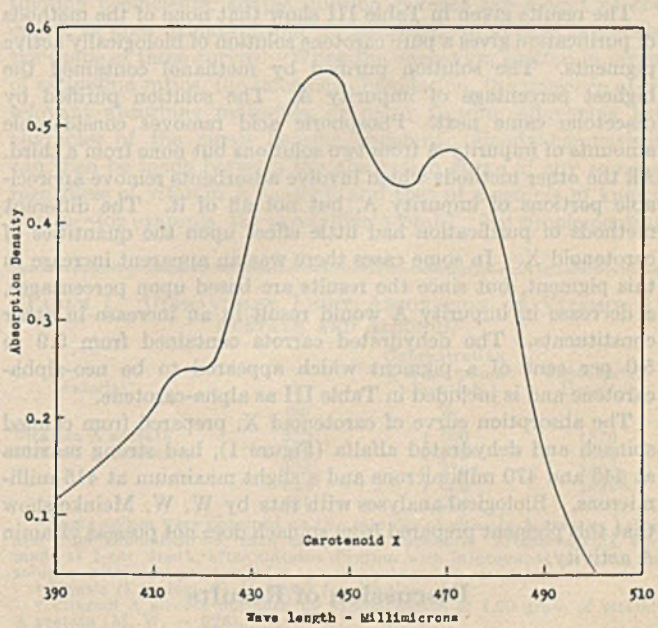


FIGURE 1. ABSORPTION CURVE OF CAROTENOID X IN PETROLEUM NAPHTHA

Table III. Constituents of Carotene Solutions Obtained by Different Methods

Method of Purification	Crude Carotene	Loss in $Ca(OH)_2$ Columns	Constituents: Impurity A	Carotenoid X	Beta-carotene	Neo-beta-carotene	Alpha-carotene
	P. p. m.	%	%	%	%	%	%
Alfalfa, dehydrated							
A. Methanol	31.7	7.9	30.8	26.1	37.5	5.6	...
B. Diacetone	31.0	10.3	20.1	...	70.5	9.4	...
C. Methanol and shaking with X reagent	24.0	5.8	16.3	30.2	46.0	7.5	...
D. $MgCO_3$ column	23.0	0.9	18.4	30.7	41.3	9.6	...
E. $CaHPO_4$ column	22.0	1.8	12.5	34.3	44.4	8.8	...
F. MgO + Supercel column	22.0	15.8	9.2	19.8	60.3	10.7	...
G. 85% H_3PO_4	27.6	7.3	33.8	15.5	29.1	21.6	...
Alfalfa, dehydrated							
A. Methanol	105.0	10.5	14.9	20.2	40.8	18.1	...
B. Diacetone	96.0	9.9	12.1	19.7	55.5	12.7	...
C. Methanol and shaking with X reagent	88.0	3.4	4.7	15.3	60.0	20.0	...
D. $MgCO_3$ column	77.0	−0.6	1.3	13.6	60.0	24.5	...
E. $CaHPO_4$ column	77.0	8.4	2.1	14.9	52.5	30.5	...
F. MgO + Supercel column	71.0	8.8	8.3	23.8	47.7	20.2	...
G. 85% H_3PO_4	83.5	2.4	5.5	21.4	49.2	23.9	...
Canned spinach							
A. Methanol	38.8	2.6	25.1	21.6	35.4	17.3	0.6
B. Diacetone	40.4	9.4	15.8	31.7	37.2	13.7	1.6
C. Methanol and shaking with X reagent	37.5	1.1	5.1	21.5	53.2	18.0	2.2
D. $MgCO_3$ column	28.8	3.4	1.1	29.8	45.8	22.8	0.5
E. $CaHPO_4$ column	34.4	4.1	5.1	21.8	44.9	26.1	2.1
F. MgO + Supercel column	36.2	5.8	1.8	22.9	51.6	19.9	3.8
G. 85% H_3PO_4	29.5	9.2	3.0	23.9	47.7	25.4	...
Canned pumpkin							
A. Methanol	25.8	2.7	19.9	...	46.6	9.6	23.9
B. Diacetone	26.2	10.3	12.0	16.2	30.2	19.4	22.2
C. Methanol and shaking with X reagent	22.8	0.0	4.4	5.7	47.3	7.5	35.1
D. $MgCO_3$ column	23.2	1.7	3.5	7.5	43.8	11.9	33.3
E. $CaHPO_4$ column	20.6	−2.9	1.9	6.1	48.2	13.7	30.1
F. MgO + Supercel column	21.8	3.7	5.7	11.4	40.9	17.3	24.7
Dehydrated carrots							
A. Methanol	844.0	10.6	3.9	...	63.4	4.9	27.8
B. Diacetone	840.0	17.4	5.3	...	59.6	5.8	29.3
C. Methanol and shaking with X reagent	853.0	12.9	2.7	...	63.7	4.5	29.1
D. $MgCO_3$ column	783.0	5.1	2.1	...	62.3	4.2	31.4
E. $CaHPO_4$ column	763.0	5.5	2.6	...	60.9	6.3	30.2
F. MgO + Supercel column	876.0	6.1	1.9	...	61.9	5.5	30.7

washing the column with petroleum ether containing 3 to 5 per cent acetone. The eluate was washed with water, concentrated in a vacuum, and subjected to chromatographic analysis. In method G the carotenoid extract was shaken with 85 per cent orthophosphoric acid [Haagen-Smit, Jeffreys, and Kirchner (7)].

The results given in Table III show that none of the methods of purification gives a pure carotene solution of biologically active pigments. The solution purified by methanol contained the highest percentage of impurity A. The solution purified by diacetone came next. Phosphoric acid removes considerable amounts of impurity A from two solutions but none from a third. All the other methods which involve adsorbents remove appreciable portions of impurity A, but not all of it. The different methods of purification had little effect upon the quantities of carotenoid X. In some cases there was an apparent increase in this pigment, but since the results are based upon percentages, a decrease in impurity A would result in an increase in other constituents. The dehydrated carrots contained from 0.9 to 5.0 per cent of a pigment which appeared to be neo-alpha-carotene and is included in Table III as alpha-carotene.

The absorption curve of carotenoid X, prepared from canned spinach and dehydrated alfalfa (Figure 1), had strong maxima at 445 and 470 millimicrons and a slight maximum at 415 millimicrons. Biological analyses with rats by W. W. Meinke show that this pigment prepared from spinach does not possess vitamin A activity.

Discussion of Results

All methods for the determination of carotene gave solutions containing beta-carotene, neo-beta-carotene, impurity A, carotenoid X, and in a few cases alpha-carotene and neo-alpha-carotene. The relative proportions of the ingredients depended upon the material used and the method of purification. No method of purification had an appreciable effect upon the quantity of carotenoid X, which appears to be so closely related to beta-carotene that, like neo-beta-carotenes, it is separated only by chromatographic analysis with calcium hydroxide. The fact that carotenoid X lacks vitamin A potency makes it highly significant in evaluating the vitamin A activity of materials from carotene analyses. The neo-beta-carotene found is not so significant because according to biological tests with rats by W. W. Meinke in this laboratory, it has one half the potency of beta-carotene. Impurity A is also significant, in that it has very little, if any, vitamin A activity (*15*).

Adsorption on calcium hydroxide is a method available for the separation and determination of the various carotenes in the mixture obtained in the analysis of plant materials. This method, however, requires much time. There are some losses in the calcium hydroxide column which can be reduced by an experienced worker. For rapid control work, it will probably be desirable to determine crude carotene by a convenient method which at the same time gives values that closely approximate the actual amounts of carotenes which possess vitamin A activity. This crude carotene will contain colored impurities, and will also frequently contain carotenes which have lower biological values than beta-carotene. The carotene solutions secured from plants by any method of determination heretofore proposed, except chromatographic separation with calcium hydroxide, usually contain three or more yellow pigments, analysis by these methods must be recognized as giving only approximate results.

Literature Cited

(1) Assoc. Official Agr. Chem., Official and Tentative Methods of Analyses, 5th ed., p. 369 (1940).
(2) Beadle, B. W., and Zscheile, F. P., *J. Biol. Chem.*, 144, 21 (1942).
(3) Fraps, G. S., and Kemmerer, A. R., Ind. Eng. Chem., Anal. Ed., 13, 806 (1941).
(4) Fraps, G. S., and Kemmerer, A. R., *J. Assoc. Official Agr. Chem.*, 22, 190 (1939).
(5) Fraps, G. S., and Kemmerer, A. R., *News Ed. (Am. Chem. Soc.)*, 19, 846 (1941).
(6) Gillam, A. E., El Ridi, M. S., and Kon, S. K., *Biochem. J.*, 31, 1605 (1937).
(7) Haagen-Smit, A. J., Jeffreys, C. E. P., and Kirchner, J. C., Ind. Eng. Chem., Anal. Ed., 15, 179 (1943).
(8) Hegsted, D. M., Porter, J. W., and Peterson, W. H., *Ibid.*, 11, 256 (1939).
(9) Kemmerer, A. R., *J. Assoc. Official Agr. Chem.*, 24, 859 (1941).
(10) *Ibid.*, 25, 886 (1942).
(11) *Ibid.*, 26, 77 (1943).
(12) Moore, L. A., Ind. Eng. Chem., Anal. Ed., 12, 726 (1940).
(13) Polgár, A., and Zechmeister, L., *J. Am. Chem. Soc.*, 64, 1856 (1942).
(14) Wall, M. F., and Kelly, E. G., Ind. Eng. Chem., Anal. Ed., 15, 18 (1943).
(15) Wiseman, H. G., Kane, E. A., Shinn, L. A., and Cary, C. A., *J. Agr. Research*, 57, 635 (1938).
(16) Zechmeister, L., and Tuzson, P., *Biochem. J.*, 32, 1305 (1938).

Chemical and Physical Determinations of Vitamin A in Fish Liver Oils

BERNARD L. OSER, DANIEL MELNICK, AND MORTON PADER
Food Research Laboratories, Inc., Long Island City, N. Y.

The results of a comparative study of spectrophotometric and colorimetric (antimony trichloride) methods for the estimation of vitamin A in fish liver oils are reported. An improved method for plotting the ultraviolet absorption curves of vitamin A products is presented, and applied in studies of crystalline vitamin A acetate, fish liver oils, and concentrates to evaluate factors which cause distortions in the curves. Emphasis is placed on the importance of conducting the determination on the unsaponifiable fraction of oils regardless of their potency. A method is described for extracting the unsaponifiable fraction without the loss of vitamin or the introduction of irrelevant absorbing materials. The U. S. P. reference cod liver oil No. 2 is shown to be unsuited as a spectrophotometric or colorimetric standard.

THE biological vitamin A activity of food or pharmaceutical products may result from the presence of a multiplicity of substances, including preformed vitamin A, either as the free alcohol or as esters, and various carotenoid pigments, of which the principal one is β-carotene. Obviously, therefore, the estimation by chemical or physical means of the vitamin A potency of a product may be complicated to a degree dependent on the nature and number of compounds present having vitamin A activity in vivo. A further factor outside the scope of nonbiological methods is the rate and degree of absorption from the intestinal tract of the various forms of vitamin A. This is dependent upon the cellular structure, digestibility, and fat and tocopherol content of the food, and is affected by the presence of nonabsorbable oils. Thus the true vitamin A potency of a food can be measured only biologically, whereas chemical or physical methods yield an estimate of the total content of vitamin A or its precursors.

The vitamin A potency of fish liver oils can be readily determined by spectrophotometric and colorimetric methods of assay, because in these oils the vitamin occurs almost exclusively as preformed vitamin A and is present in comparatively high concentrations.

Ultraviolet Absorption Curve of Free and Esterified Vitamin A

The ultraviolet absorption curve of pure vitamin A has appeared in several recent reports (*2*, *19*). In Figure 1 are plotted the critical parts of the curves (from 300 to 350 millimicrons) for isopropanol solutions of vitamin A acetate (obtained from Distillation Products, Rochester, N. Y.) and the alcohol prepared therefrom. For reasons stated below it is desirable to plot the results in terms of extinction ratios ($E_{\lambda/328}$) rather than extinction coefficients ($E^{1\%}_{1\ cm.}$ 328 mμ). Another pure vitamin A preparation, the distilled vitamin A esters (from Distillation Products) in corn oil, has been found to yield practically the same curves before and after saponification (*11*).

The Beckman quartz spectrophotometer (manufactured by National Technical Laboratories, South Pasadena, Calif.), calibrated against the lines of the mercury spectrum, was used for the spectrophotometric measurements. To determine the reproducibility of this instrument a series of glass disks, optically ground and having graded absorption properties, was prepared and distributed among various laboratories which were requested to measure their extinction coefficients at 328 millimicrons. The results of these tests indicated a high degree of reproducibility for the Beckman instrument, the coefficient of variation of the average deviations being of the order of 1 per cent. A recent study of several spectrophotometers used for assaying vitamin A oils (*16*) likewise showed excellent reproducibility in the Beckman measurements.

After saponification of the vitamin A ester a slight shift towards the lower wave lengths occurs in the entire absorption curve, so that the absorption maximum for the alcohol is at 325 rather than 327 millimicrons. This phenomenon has been observed not only with the pure compound, but also with naturally occurring fish liver oils. Another point of interest, illustrated in Table I, is the higher extinction coefficient at 325, or even 328 millimicrons, of an equivalent amount of the alcohol (prepared by saponification of the acetate) as compared with that of the acetate. Similar observations have been reported in studies on fish liver oils when the measurements were made in polar solvents, in which the unsaponifiable fraction gave a higher extinction coefficient than in nonpolar solvents (*1*, *8*). This was not so pronounced when the determinations were made on the whole oils, where the vitamin occurs esterified.

In Figure 1, and in all the absorption curves presented, emphasis is placed on the qualitative nature or shape of the absorption curve rather than on the absorption intensity or position on the vertical axis as determined by the concentration. The ordinate represents the extinction ratio—that is, the ratio of the extinction coefficient (or photometric density) at a given wave length to that at 328 millimicrons, the generally accepted absorption maximum for naturally occurring vitamin A esters. In order to demonstrate the advantages of this method of plotting the absorption curve, the same data for vitamin A acetate after oxidation and after dilution are plotted in Figure 2 in terms of extinction coefficients and extinction ratios. When plotted in terms of extinction coefficients, simple dilution reduces the height of the curve, as does oxidation, but no noticeable difference is seen in the shape of these curves. When the curves are plotted in terms of extinction ratios, the influence of oxidation, the presence of irrelevant absorbing materials, or other factors influencing the character of the curve become apparent. Ready comparison can then be made between the absorption curve of any sample, regardless of vitamin A content, and that of a sample of known purity—for example, the crystalline acetate. By supplementing

TABLE I. ULTRAVIOLET LIGHT ABSORPTION OF VITAMIN A ACETATE AND ALCOHOL[a]

Material	λ	Concentration in Isopropanol Solution	Photometric Density[b]
	mμ	%	
Vitamin A acetate	328	1.000	1570
Vitamin A alcohol	328	0.872[c]	1590
	325	0.872[c]	1620
	328	1.000	1825[d]
	325	1.000	1860[d]

[a] Same values were obtained on two samples of vitamin A acetate, before and after saponification, each assayed in duplicate. Measurements were made at 1-cm. depth, after suitable dilution with isopropanol to bring test solution within proper range for spectrophotometric measurement.
[b] Equals (2 − log₁₀ G) × factor for dilution.
[c] Vitamin A alcohol obtained by saponification of 1.00 gram of vitamin A acetate (M. W. = 328).
[d] Expressed in terms of 1.000 gram of vitamin A alcohol derived from 1.146 grams of acetate.

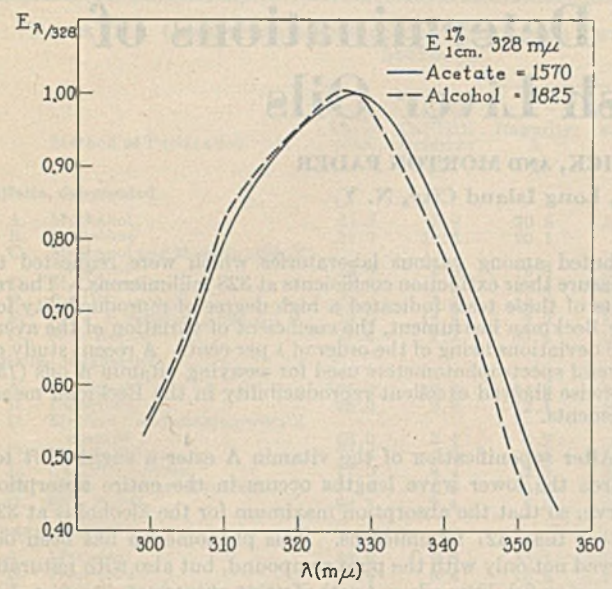


FIGURE 1. ULTRAVIOLET ABSORPTION CURVES VITAMIN A ACETATE AND VITAMIN A ALCOHOL IN ISOPROPANOL

the curve with the extinction coefficient at 328 millimicrons (the *E* value), the pertinent data for interpreting the spectrophotometric measurements are given.

Reaction of Vitamin A in Fish Liver Oils with Antimony Trichloride

This reaction measures vitamin A both as the ester and free alcohol. When the test is applied to fish liver oils directly, especially to low-potency oils, inhibitors of the color development are often encountered and must be corrected for by use of the increment procedure (*11*). Freshly opened samples of U. S. P. reference cod liver oil No. 2 appear to have large amounts of inhibitors. Upon saponification the inhibitors are removed and the vitamin develops its full color intensity with the reagent, as shown by a normal increment in photometric density for the 10 units of vitamin A added to an aliquot of the final test solution. In addition, nonspecific, saponifiable materials are present in fish liver oils which react with antimony trichloride to form a blue color absorbing light at 620 millimicrons. For these reasons it is preferable to use the unsaponifiable fraction rather than whole oil for test purposes.

Changes in the Absorption Curve of Vitamin A on Oxidation

Investigations of the oxidative destruction of vitamin A in fish liver oils, using various oxidation procedures and assay methods, have been reported (*6*, *7*, *13*, *14*, *17*). The results of a typical experiment to determine the effect of oxidation upon the ultraviolet absorption curve of crystalline vitamin A are presented in Figure 3.

Solutions of vitamin A acetate and vitamin A alcohol (obtained by saponification of the acetate) in ethyl laurate were vigorously aerated in parallel at 45° C., in the absence of light, in an all-glass apparatus. Samples were removed at intervals and their vitamin A content was determined spectrophotometrically and colorimetrically. In testing such pure solutions the precision of the two assay procedures is good; the spectrophotometric values are reproducible within ±1 per cent, the colorimetric within ±2 per cent. The air was passed through pure ethyl laurate, also at 45° C., before entering the test solutions, the diluent so aerated being used as a blank in the spectrophotometric measurements. Ethyl laurate is ideally suited for this type of experiment, since it absorbs light to a negligible extent in the range of 295 to 355 millimicrons, undergoes no optical changes upon aeration, and does not interfere with the color reaction with antimony trichloride, before or after aeration.

In the cases of both the alcohol and acetate the changes in the curve were limited almost entirely to the region below 328 millimicrons, the extinction ratios of the samples at the lower wave lengths increasing progressively during the period of oxidation. Robinson (*13*), in tests conducted with a fish liver concentrate rather than the pure vitamin, obtained similar results in so far as the spectrophotometric data are concerned. This is not readily apparent in his paper, since his curves were plotted in terms of extinction coefficients.

In these tests the inability of the antimony trichloride reagent to differentiate between oxidized and nonoxidized vitamin A is demonstrated. At first, there is fairly good agreement between the spectrophotometric and colorimetric estimates, as would be expected when working with pure solutions of vitamin A. [For comparison the colorimetric values are expressed as "vitamin A units per gram ÷ 2000", since the spectrophotometric estimates are derived from *E* value × 2000 (the commercial standard conversion factor).] During the initial stages of oxidation there is a tendency for the colorimetric values to be somewhat less than the *E* values. The slightly lower values obtained colorimetrically for the freshly prepared solutions may be due to small amounts of oxidized vitamin in the crystalline preparation. Large losses of vitamin A by oxidation must occur before significant distortions in the ultraviolet absorption curves become apparent. After prolonged aeration of the solutions, the values determined by the antimony trichloride reaction became equal to and then significantly greater than those obtained spectrophotometrically. Estimates of residual nonoxidized vitamin A based on the *E* values of the aerated solutions would, from the very nature of the absorption curves, be much too high. Colorimetric estimates approximating these values would thus also be in error. These observations do not agree with those of Robinson (*13*), who found that as the oxidation progressed the relative differences

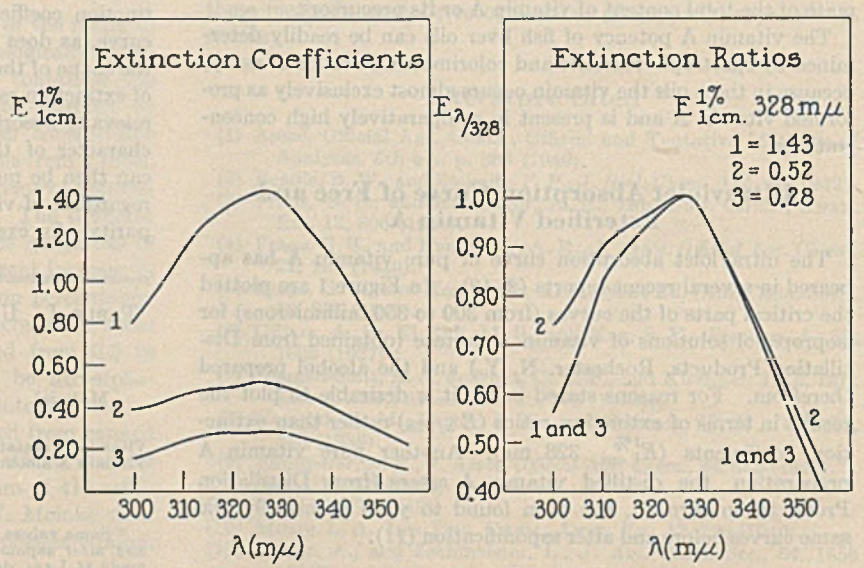


FIGURE 2. ADVANTAGE OF PLOTTING EXTINCTION RATIOS RATHER THAN EXTINCTION COEFFICIENTS IN PRESENTING ULTRAVIOLET ABSORPTION CURVES OF VITAMIN A SOLUTIONS

1. Vitamin A acetate in ethyl laurate
2. Oxidation of (1) by air
3. Dilution of (1) 1 to 5 with ethyl laurate

Table II. Ultraviolet Absorption Maximum of Samples of Fish Liver Oils[a]

(Fish liver oils taken at random from a series of test samples; values are not necessarily representative of each species.)

Sample	$E^{1\%}_{1\,cm.}$ 328 mμ	Maximum mμ	$E^{1\%}_{1\,cm.}$ Max.	Difference[b] $E^{1\%}_{1\,cm.}$ 328 mμ − $E^{1\%}_{1\,cm.}$ Max. %
Vitamin A acetate	1570	327	1570	0.0
Same, after saponification	1590	325	1620	−1.9
Distilled vitamin A esters	107.8	328	107.8	0.0
Same, unsaponifiable fraction	106.3	325	107.0	−0.7
Cod liver oil (No. 1)	0.87	320	0.94	−7.4
Same, unsaponifiable fraction	0.71	325	0.72	−1.4
Cod liver oil (No. 2)	1.58	328	1.58	0.0
Cod liver oil (No. 3)	1.14	320	1.27	−10.2
Same, unsaponifiable fraction	0.98	325	0.99	−1.0
Dogfish liver oil	6.04	328	6.04	0.0
Shark liver oil	8.02	326	8.11	−1.1
Soupfin shark liver oil	56.0	328	56.0	0.0
Black cod liver oil	55.2	328	55.2	0.0
Ling cod liver oil	94.1	328	94.1	0.0
Halibut liver oil	29.5	327	30.0	−1.6
Tuna liver oil	60.1	327	60.1	0.0
Concentrates (prepared by saponification)	56.7	326	56.9	−0.4
	96.1	327	97.2	−1.1
	498	325	508	−2.0

[a] In isopropanol solution.

[b] Value for $E^{1\%}_{1\,cm.}$ maximum is taken as basis for comparison.

between the spectrophotometric and colorimetric values increased, the colorimetric values always being lower.

As the oxidation proceeded the color reaction, with antimony trichloride became increasingly atypical. The rate of color development decreased; fully 30 seconds were required to reach maximal color intensity in the sample aerated for 140 hours, as compared with 4 seconds in the case of the non-oxidized solution. In measuring the color developed, the 4-second galvanometer reading was used rather than that corresponding to maximal color development. When the latter was used low values were obtained for added increments of vitamin A, resulting in extremely high vitamin A estimates for samples containing oxidized vitamin A.

The above colorimetric and spectrophotometric tests suggest that there may be more than one form of oxidized vitamin A, those first produced reacting to a lesser extent than vitamin A with the antimony trichloride reagent, while other compounds formed in the later stages of oxidation react to an even greater extent than vitamin A, based upon comparable E values at 328 millimicrons. Obviously the reaction of the latter forms of oxidized vitamin A with the antimony trichloride reagent is much less than that observed for an equivalent amount of the vitamin.

Reliability of Spectrophotometric Method Based upon a Single Reading at 328 Millimicrons

In determining the vitamin A potency of fish liver oils, there is, as yet, no complete agreement as to the wave length at which the extinction coefficient of the oil should be measured. The absorption maximum of vitamin A alcohol in polar solvents (such as isopropanol) is now known to be closer to 325 than to 328 millimicrons. However, fish liver oils may exhibit other absorption maxima in the critical region of the spectrum.

In Table II are presented extinction coefficients at both the maximum and 328 millimicrons of several fish liver oils chosen at random from a large series of samples. Excluding atypical oils which show absorption maxima far from that of vitamin A (usually low-potency oils), the samples are seen to have maxima ranging from 325 to 328 millimicrons in isopropanol. The unsaponifiable extracts of these same samples invariably exhibited maximal absorption at 325 millimicrons. It is apparent that, in fish liver oils with absorption maxima in the region of 325 to 328 millimicrons, the accuracy of a determination is not seriously affected by using the extinction coefficient at 328 millimicrons in preference to that at the maximum, or vice versa. The difference is of the order of 1 to 2 per cent. When the absorption maximum of an oil departs significantly from the 325 to 328 millimicron range, the presence of appreciable quantities of irrelevant absorbing material is indicated and vitamin A estimates based upon such a maximum are in error.

It is the practice in these and most other laboratories to evaluate the vitamin A content of fish liver oils on the basis of E values—i. e., the extinction coefficient at 328 millimicrons. It has been reasonably suggested that, in the case of unsaponifiable concentrates of fish liver oils, the extinction coefficient at 325 millimicrons be used. However, many such concentrates are frequently diluted with whole fish liver oils, a practice which introduces complications in assaying such materials. In any case, the use of the 328-millimicron

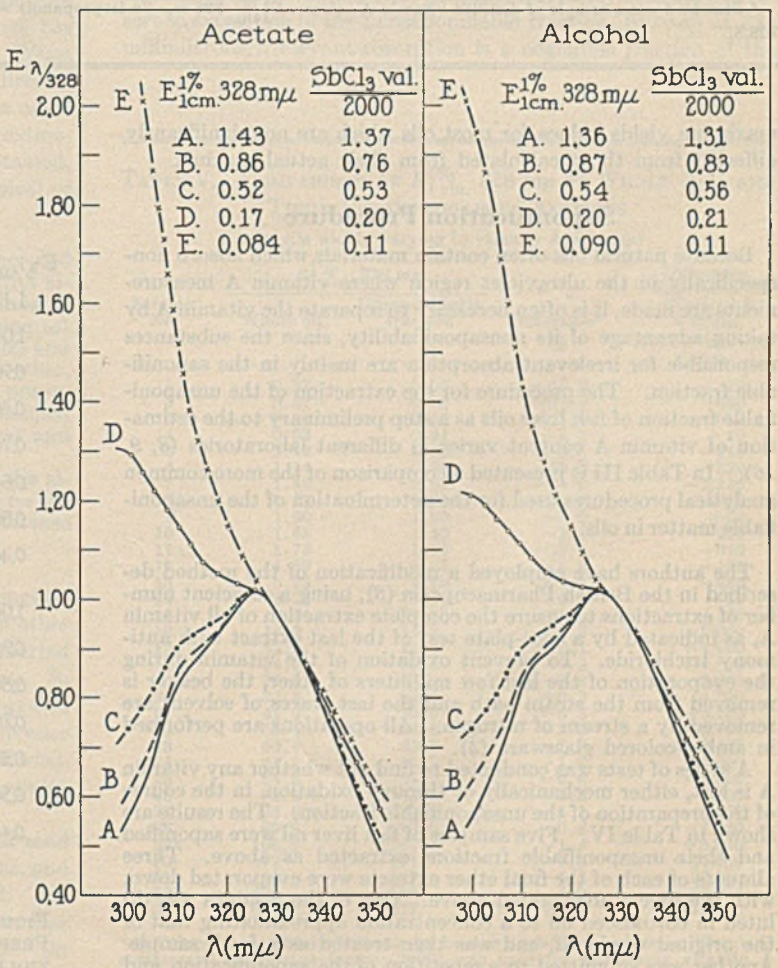


Figure 3. Changes in Absorption Curves of Vitamin A Acetate and Alcohol on Oxidation

Aerated at 45° C. *A*, 0 hour; *B*, 24 hours; *C*, 48 hours; *D*, 90 hours; *E*, 140 hours

TABLE III. EXTRACTION OF UNSAPONIFIABLE FRACTION OF FISH LIVER OILS

(Step-by-step comparison of three methods)

Step	United States Pharmacopoeia XII (*12*)	Kerr-Sorber (Modified, *5*)	British Pharmacopoeia (Modified, *3*)
Saponification	Reflux 5 grams of oil in 40 ml. of 1.1 *N* alcoholic (95%) KOH for 120 minutes	Reflux 5 grams of oil in 35 ml. of 1.8 *N* alcoholic (86%) KOH for 20 minutes	Reflux 0.2 to 1 gram of oil in 15 ml. of 0.5 *N* alcoholic (95%) KOH for 15 minutes
Extraction	Evaporate the alcohol Take up the residue in 50 ml. of water Extract twice with 50 ml. of ether	Cool to 30° C. Extract once with 150 ml. of ether	Add 30 ml. of water Extract 4 to 5 times with 30 ml. of ether (Test last extract with $SbCl_3$)
Washing	Once with 20 ml. of 0.1 *N* NaOH Once with 20 ml. of 0.2 *N* NaOH Then with 20-ml. portions of water until the last washing gives no color with phenolphthalein	3 times with 100 ml. of 0.2 *N* KOH Then with 30-ml. portions of water until last washing gives no color with phenolphthalein	Once with 30 ml. of water Once with 15 ml. of 0.5 *N* KOH Then with 30-ml. portions of water until last washing gives no color with phenolphthalein
Evaporation	To dryness on a steam bath	To dryness on a steam bath	To *ca.* 20 ml. on a steam bath Remove from steam bath, and blow off the remainder of the solvent (do not bring to complete dryness) with a stream of nitrogen

TABLE IV. RELIABILITY OF PROCEDURE FOR PREPARATION OF UNSAPONIFIABLE EXTRACT OF FISH LIVER OILS

Sample No.[a]	Procedure for Estimating Vitamin A	Vitamin A Content, U. S. P. Units per Gram: Whole sample, *a*	Unsaponifiable fraction, *b*	Resaponification: *b* alone	Resaponification: *b* + cottonseed oil
16	Spectrophotometric[b]	32,400	30,400	31,500	32,760
	$SbCl_3$ reaction[c]	32,300	29,600	32,200	30,500
18	Spectrophotometric	49,400	44,800	44,100	44,300
	$SbCl_3$ reaction	50,800	39,100	39,300	39,900
19	Spectrophotometric	52,000	50,000	49,600	49,600
	$SbCl_3$ reaction	52,900	42,800	43,100	47,200
22	Spectrophotometric	117,000	113,600	112,400	112,400
	$SbCl_3$ reaction	119,000	110,000	108,000	105,000
23	Spectrophotometric	120,800	111,400	114,500	114,500
	$SbCl_3$ reaction	122,000	117,000	117,000	114,000

[a] Numbers correspond to those in Table V.

[b] Commercial standard conversion factor of 2000 was used for estimating vitamin A content from $E^{1\%}_{1cm.}$ 328 mμ.

[c] Standard was a sample of distilled vitamin A esters; $E^{1\%}_{1cm.}$ 328 mμ (in isopropanol) = 108.5.

maximum yields values for most oils which are not significantly different from those calculated from their actual maxima.

Saponification Procedure

Because natural oils often contain materials which absorb nonspecifically in the ultraviolet region where vitamin A measurements are made, it is often necessary to separate the vitamin A by taking advantage of its nonsaponifiability, since the substances responsible for irrelevant absorption are mainly in the saponifiable fraction. The procedure for the extraction of the unsaponifiable fraction of fish liver oils as a step preliminary to the estimation of vitamin A content varies in different laboratories (*8*, *9*, *16*). In Table III is presented a comparison of the more common analytical procedures used for the determination of the unsaponifiable matter in oils.

The authors have employed a modification of the method described in the British Pharmacopoeia (*3*), using a sufficient number of extractions to ensure the complete extraction of all vitamin A, as indicated by a spot-plate test of the last extract with antimony trichloride. To prevent oxidation of the vitamin during the evaporation of the last few milliliters of ether, the beaker is removed from the steam bath and the last traces of solvent are removed by a stream of nitrogen. All operations are performed in amber-colored glassware (*4*).

A series of tests was conducted to find out whether any vitamin A is lost, either mechanically or through oxidation, in the course of the preparation of the unsaponifiable fraction. The results are shown in Table IV. Five samples of fish liver oil were saponified and their unsaponifiable fractions extracted as above. Three aliquots of each of the final ether extracts were evaporated down, with the precautions stated above. One of the residues was diluted in cottonseed oil to a concentration approximating that of the original whole oil, and was then treated as a fresh sample. Another was submitted to a repetition of the saponification and extraction procedures without the addition of cottonseed oil. The third residue was taken up in isopropanol directly and its extinction coefficient at 328 millimicrons measured.

If loss of vitamin A had occurred due to incomplete extraction or to oxidation, the vitamin A content of the second unsaponifiable fraction would be expected to be lower than that of the original unsaponifiable fraction. That such was not the case, however, may be seen in the good agreement for each sample among all three spectrophotometric estimates based on the unsaponifiable fractions. Similarly close agreement may also be observed where the vitamin content was determined by the antimony trichloride method. (Both the spectrophotometric and colorimetric tests were performed using aliquots of the same final ether extract.) A comparison of the ultraviolet absorption curves of the unsaponifiable fractions within a set showed no differences, a further indication that no substantial oxidative changes had occurred.

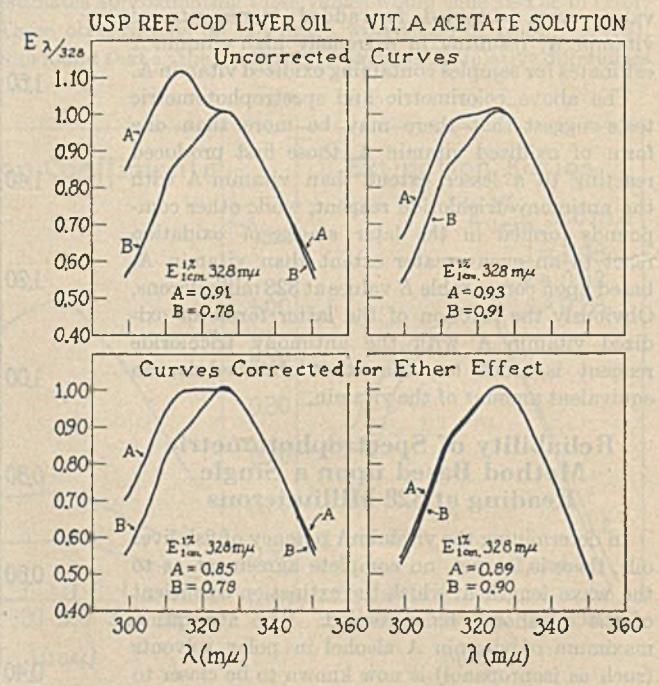


FIGURE 4. INFLUENCE OF IMPURITIES IN ETHER USED IN PREPARATION OF UNSAPONIFIABLE EXTRACTS ON ULTRAVIOLET ABSORPTION CURVES AND EXTINCTION COEFFICIENTS OF VITAMIN A ALCOHOL SOLUTIONS

A. Preparation of unsaponifiable extract with U. S. P. ether
B. Preparation of unsaponifiable extract with redistilled U. S. P. ether

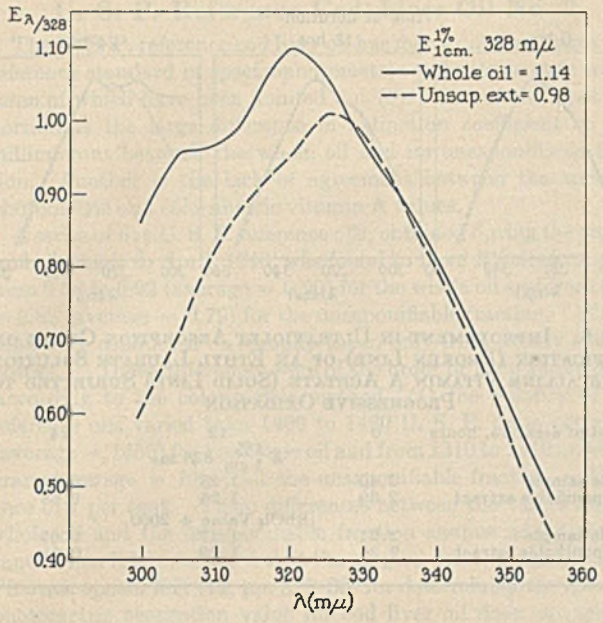


FIGURE 5. REMOVAL OF IRRELEVANT LIGHT-ABSORBING MATERIALS IN A COD LIVER OIL BY SAPONIFICATION

Increasing specificity of spectrophotometric method for determination of vitamin A

The necessity for the use of specially prepared alcohol for the saponification procedure and specially prepared ether for the subsequent extraction was investigated. The general practice has been to use aldehyde-free alcohol and peroxide-free ether. When alcohol (S. D. No. 30) and ether (U. S. P. XII) were used directly from their commercial containers in the preparation of the unsaponifiable extracts of low potency oils, erroneously high extinction coefficients at 328 (or 325) millimicrons were obtained. Furthermore, the ultraviolet absorption curves were atypical of vitamin A alcohol.

A series of assays was conducted on the U. S. P. reference cod liver oil No. 2 on pure vitamin A acetate in 95 per cent ethyl alcohol (aldehyde-free), and on a sample of distilled vitamin A esters. A suitable sized sample of each was saponified using (*a*) redistilled alcohol (over aluminum and potassium hydroxide) and nonredistilled ether, (*b*) redistilled alcohol and redistilled ether, (*c*) nonredistilled alcohol and nonredistilled ether, and (*d*) nonredistilled alcohol and redistilled ether. The ether was redistilled without the addition of any reagents, discarding the first and last 10 per cent of the distillate.

The completeness of the removal of the aldehydes from the alcohol was verified by the absence of any color development on the addition of fuchsin-sulfite reagent (*18*) which produced an intense red color with the untreated solvent.

The use of nonredistilled alcohol affected neither the extinction coefficient nor the shape of the absorption curve. On the other hand, the use of nonredistilled ether resulted in both a distorted curve and a high extinction coefficient at 328 millimicrons. In Figure 4 (upper two sets of curves) are presented the absorption curves of the unsaponifiable fractions of the U. S. P. reference cod liver oil and that prepared from an ethanol solution of vitamin A acetate. The effect of using nonredistilled ether is apparent.

To correct for the ether effect, the same volume of ether used in the above tests was evaporated under the same conditions, and the residue was dissolved in the same volume of isopropanol. The absorption curve was then obtained. A similar correction was made for the redistilled ether, but this was extremely small. On applying these corrections the lower set of curves in Figure 4 was obtained. In the case of the vitamin A acetate both curves became superposable, indicating that the vitamin A itself was not affected by the impurities in the ether, which are carried through the saponification procedure to the final solution. In the case of the U. S. P. oil, only partial improvement was noted. Apparently, substances in this oil other than vitamin A had been affected.

The ultraviolet absorption curves (and likewise the extinction coefficients at 328 millimicrons) of the unsaponifiable extracts of the distilled vitamin A esters were the same regardless of which set of solvents was used. This is undoubtedly due to simple dilution of the nonspecific absorbing substances in the ether when adjusting the vitamin A concentration of the test solution to the proper range for the photometric measurements. In this case also the vitamin A was not affected.

Necessity for Determining the Vitamin A Content in the Unsaponifiable Fraction

Large errors may be made in the determination of the vitamin A content of fish liver oils when the estimates are based on a whole oil rather than on its unsaponifiable fraction. It is generally agreed that in the low-potency ranges these differences are significant. In Figure 5 is illustrated the change in the ultraviolet absorption curve in isopropanol of a sample of cod liver oil after saponification. The whole oil has two maxima, neither of which is characteristic of vitamin A. Furthermore, the extinction coefficient at 328 millimicrons is 16 per cent higher for the whole oil than for its unsaponifiable fraction.

On the other hand, it is not so commonly recognized that differences of equally important magnitude may be observed even in high-potency oils. Morton (*10*) states, "In the case of oils containing more than 10,000 I. U. per gram it is rarely necessary to resort to extraction of the nonsaponifiable fraction, because at 325 millimicrons, irrelevant absorption is a negligible fraction of the

TABLE V. COMPARISON OF $E^{1\%}_{1\,cm.}$ 328 mμ OF WHOLE OILS AND THEIR UNSAPONIFIABLE FRACTIONS

(In oils widely varying in vitamin A potency)

Sample No.	$E^{1\%}_{1\,cm.}$ 328 mμ		Difference[a]	Difference Expressed as Vitamin A Unitage
	Whole oil	Unsaponifiable fraction	%	*U. S. P. units per gram*
1	0.23	0.07	−68.2	−320
2	0.87	0.53	−39.8	−680
3	0.91	0.57	−37.9	−680
4	0.93	0.91	−2.2	−40
5	1.12	1.05	−6.5	−140
6	1.14	0.98	−14.0	−320
7	1.49	0.87	−41.6	−1,240
8	1.51	1.20	−20.8	−620
9	1.60	1.63	+2.0	+60
10	1.65	1.52	−7.6	−260
11	1.75	1.43	−18.6	−640
12	4.89	4.65	−4.9	−480
13	5.73	5.59	−2.4	−280
14	8.25	8.26	+0.1	+20
15	9.44	9.53	+1.0	+180
16	16.2	15.2	−6.2	−2,000
17	23.9	21.5	−10.1	−4,800
18	24.7	22.4	−9.3	−4,600
19	26.0	25.0	−3.8	−2,000
20	32.5	32.6	+0.3	+200
21	41.9	39.3	−6.3	−5,200
22	58.5	56.8	−2.9	−3,400
23	60.4	55.7	−7.8	−9,400
24	61.7	60.7	−1.7	−2,000
25	64.3	59.9	−6.8	−8,800
26	72.8	69.9	−3.9	−5,800
27	96.6	92.6	−4.1	−8,000
28	99.8	99.0	−0.8	−1,600
29[b]	108.5	107	−1.4	−3,000
30	179	171	−4.5	−16,000
31	198	187	−5.5	−22,000
32	606	580	−4.3	−52,000
33	658	617	−6.2	−82,000

[a] Values obtained for whole oils are used as basis for comparison. Standard commercial conversion factor (2000) was used for estimating vitamin A content from $E^{1\%}_{1\,cm.}$ 328 mμ.

[b] Distilled vitamin A esters.

observed value. If, however, the color test appears anomalous, or does not agree with the ultraviolet assay, it is always safer to work on the nonsaponifiable fraction." However, even when good agreement by both methods is obtained, vitamin A estimates of high-potency oils may be appreciably less when the determination is conducted on the unsaponifiable fraction. In Table V are shown the differences in spectrophotometric estimates of vitamin A content between the whole and unsaponifiable fractions of a series of oils ranging from low to high potencies. A similar tabulation, using the antimony trichloride procedure (*11*), is shown in Table VI.

In addition to greater accuracy, an increase in precision is gained by conducting the antimony trichloride test according to the method described (*11*). The average deviation in the assay of fish liver oils (unsaponifiable fractions) is ordinarily of the order of ±2 per cent, which compares favorably with that of the spectrophotometric method. This may be illustrated by the data in Table IV where, even if the more involved redeterminations of the unsaponifiable fractions be regarded as triplicate tests, the average deviation by the spectrophotometric method is found to be ±1.04 per cent, and by the colorimetric method it is ±2.19 per cent. When duplicate determinations differ by more than ±2 per cent of their mean, a redetermination is indicated.

It has already been shown that the lower values are not due to mechanical or oxidative losses in the preparation of the unsaponifiable extracts. The possibility exists, however, that vitamin A degradation products, which absorb light at 328 millimicrons, are partially or completely removed by virtue of an appreciable solubility in the aqueous solutions used in the preparation of the unsaponifiable extract. Accordingly, an investigation along these lines was carried out.

An ethyl laurate solution of vitamin A acetate was aerated according to the procedure previously described. Samples were removed at intervals and spectrophotometric and colorimetric assays were performed on the whole sample and on the unsaponifiable fraction derived therefrom. The results of this experiment are presented in Figure 6. The rate of oxidative destruction of vitamin A in this experiment was more rapid than that in the previous aeration experiment, doubtless because of the more rapid rate of aeration of the present solution of the vitamin A acetate. This, however, does not introduce a variable into the study, since the aeration rates were maintained constant within each experiment, and all comparisons were made relative to the freshly prepared solutions. In each case, the rate of aeration during the progressive stages of oxidation of any particular set or sets within a single experiment was maintained constant.

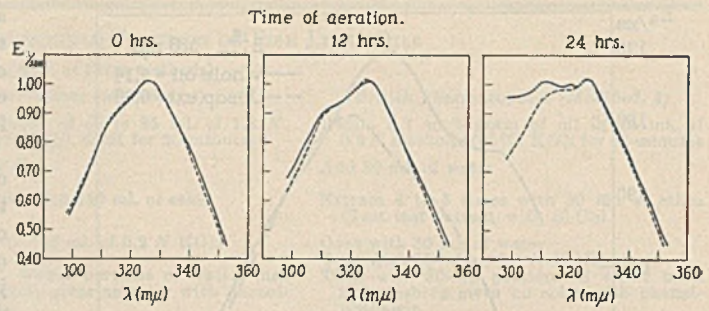


FIGURE 6. IMPROVEMENT IN ULTRAVIOLET ABSORPTION CURVE ON SAPONIFICATION (BROKEN LINE) OF AN ETHYL LAURATE SOLUTION OF CRYSTALLINE VITAMIN A ACETATE (SOLID LINE) SUBJECTED TO PROGRESSIVE OXIDATION

Period of aeration, hours	0	12	24
		$E\,{}^{1\%}_{1\,cm.}$ 328 mμ	
Whole sample	2.40	1.28	0.72
Unsaponifiable extract	2.39	1.26	0.68
		$SbCl_3$ Value + 2000	
Whole sample	2.21	1.17	0.63
Unsaponifiable extract	2.26	1.20	0.64

The data presented in Figure 6 show that the *E* value of the unsaponifiable fraction was significantly less than that of the whole sample when the latter was subjected to prolonged aeration. The initial test solution contained no appreciable amounts of irrelevant absorbing material, since it was a pure ethyl laurate solution of crystalline vitamin A acetate. Hence it appears that the oxidation products of vitamin A are partially removed in the preparation of the unsaponifiable fraction.

This is further supported by the character of the ultraviolet absorption curves obtained for the sample during progressive oxidation. Initially the curves for both the whole sample and unsaponifiable fraction corresponded with those for vitamin A acetate and vitamin A alcohol. During oxidation of the vitamin A ester the curve became progressively distorted owing to the presence of increasing amounts of vitamin A oxidation products. On the other hand, the absorption curves of the unsaponifiable fractions of the oxidized samples approached more closely that of the pure vitamin A alcohol. Obviously some oxidation products were removed in preparing the unsaponifiable extract. Since an atypical curve was still obtained with the unsaponifiable fraction it is concluded that not all of the oxidation products are removed by this procedure. Thus, estimates of the vitamin A potency of an oxidized oil (high- or low-potency) when based upon the whole sample may be erroneously high. The unsaponifiable fraction should be used for the test whenever possible.

The excellent agreement between the whole and unsaponifiable fractions of the freshly prepared vitamin A acetate solution with respect to both *E* values and colorimetric values (see legend to Figure 6) is further evidence of the reliability of the procedure used in the present study for the preparation of the unsaponifiable extract.

The excellent agreement between the colorimetric tests on the whole sample and on the unsaponifiable fraction appears at first to be somewhat at variance with the results obtained in the assay of fish liver oils (see Table VI). Apparently, the substances present in fish liver oils which react with the antimony trichloride reagent and are removed in the preparation of the unsaponifiable extracts, are not simply oxidized vitamin.

TABLE VI. COMPARISON OF VITAMIN A VALUES FOR WHOLE OILS AND THEIR UNSAPONIFIABLE FRACTIONS

(By the antimony trichloride reaction)

Sample No.[a]	$SbCl_3$ Value + 2000[b] Whole oil	Unsaponifiable fraction	Difference[c]	Difference in Vitamin A Content[c]
	Per gram		%	*U. S. P. units per gram*
5	0.91	0.88	−3.3	−60
7	1.11	0.57	−48.6	−1,080
11	1.14	1.07	−6.1	−140
16	16.2	14.8	−8.6	−2,800
17	24.4	19.6	−19.6	−9,600
18	25.4	19.6	−22.8	−11,600
19	26.5	21.4	−19.2	−10,200
20	33.9	29.8	−12.1	−8,200
22	59.5	55.0	−7.6	−9,000
23	61.0	58.5	−4.1	−5,000
25	63.0	60.0	−4.8	−6,000
26	68.5	60.5	−11.7	−16,000
27	91.5	85.0	−7.1	−13,000
29[d]	108.5	107	−1.4	−3,000
30	179	154	−14.0	−50,000
31	193	178	−7.8	−30,000
32	605	490	−19.0	−230,000
33	660	580	−12.1	−160,000

[a] Numbers correspond to those in Table V.
[b] Standard used in these tests was a sample of distilled vitamin A esters; $E\,{}^{1\%}_{1\,cm.}$ 328 mμ (in isopropanol) = 108.5. To facilitate comparisons with data presented in Table V, vitamin A values obtained colorimetrically are divided by standard conversion factor, 2000.
[c] Values obtained for whole oil are taken as basis for comparison.
[d] Distilled vitamin A esters.

U. S. P. Reference Cod Liver Oil No. 2

The U. S. P. reference cod liver oil has many disadvantages as a reference standard in spectrophotometric and colorimetric work, some of which have been pointed out (*8*). One of the most important is the large difference in extinction coefficient at 328 millimicrons between the whole oil and its unsaponifiable fraction. Another is the lack of agreement between the spectrophotometric and colorimetric vitamin A values.

A series of five U. S. P. reference oils, obtained during the period from January to April, 1943, was found to have E values ranging from 0.88 to 0.92 (average = 0.90) for the whole oil and from 0.77 to 0.82 (average = 0.79) for the unsaponifiable fraction. (These tests were conducted on samples taken only from freshly opened bottles.) These differences were of the order of 12 to 14 per cent. According to the colorimetric method (*11*) the potency of the reference oils varied from 1400 to 1480 U. S. P. units per gram (average = 1450) for the whole oil and from 1310 to 1410 units per gram (average = 1360) for the unsaponifiable fraction, a difference of 7 per cent. These differences between the values for the whole oil and the unsaponifiable fraction assume added significance when it is considered that the method described in the U. S. Pharmacopoeia XII (*12*, pp. 329–30) for determining the spectrophotometric absorption value for cod liver oil does not specify which should be taken for test purposes. Failure to include such a specification is tantamount to assuming that this relation between the absorption values for whole oil and unsaponifiable fraction is uniform for all oils, which of course is not supported by the facts.

When parallel tests on the reference oil are used as the basis for arriving at factors for converting E values to biological unitage in unknown oils, serious complications result. Errors in estimating potency in relation to the U. S. P. reference oil may fall within the limits of error of the bioassay and are difficult to prove.

Another disadvantage in the use of the U. S. P. reference oil is its chemical instability. Bottles of the oil were obtained directly from the U. S. Pharmacopoeial Convention, Philadelphia, Penna., and the spectrophotometric absorption curves of the whole oil and unsaponifiable fraction were determined before and after storage for one month at 5° C. under air or nitrogen. A typical set of curves is shown in Figure 7.

Changes not only in the extinction coefficient at 328 millimicrons but also in the actual shape of the absorption curves are noted. The distortion of the curve of the stored sample is more striking in the case of the whole oil. The upward shift of the left leg of the vitamin A curve and the decrease in extinction coefficient at the absorption maximum is typical of an oxidative change. In the test conducted on the unsaponifiable fraction it is of interest to note the slight rise in E value of the sample stored under air. This is accompanied by an upward shift of the left leg of the curve. Such a shift in the maximum and the rise in E value is not infrequently observed in the incipient stages of oxidation of an oil. Comparison of the absorption curve of the unsaponifiable fraction of the fresh sample with that of pure vitamin A indicates that even this fraction contains irrelevant absorbing material. In each series that portion of the ultraviolet absorption curves above 330 millimicrons is superposable.

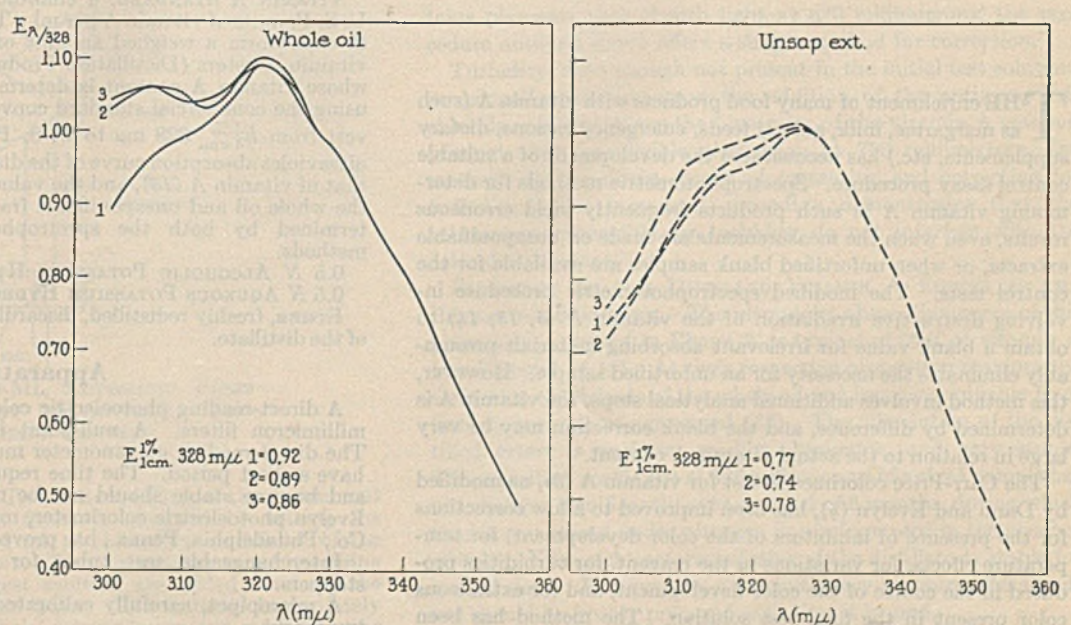


FIGURE 7. ULTRAVIOLET ABSORPTION CURVES OF U. S. P. REFERENCE COD LIVER OIL NO. 2
Before (curve 1) and after storage at 5° C. under nitrogen curve 2) and air (curve 3)

Summary

The spectrophotometric and colorimetric (antimony trichloride) methods for the determination of vitamin A in fish liver oils have been critically studied.

The ultraviolet absorption curves for crystalline vitamin A acetate and the alcohol, derived therefrom, are presented. By plotting extinction ratios rather than extinction coefficients it is possible to obtain curves for oils, regardless of vitamin A content, which lend themselves to direct comparison with that of a pure vitamin A preparation. During oxidation of vitamin A, either as the free alcohol or as the acetate, the distortion in the ultraviolet absorption curve is almost entirely in the region below the respective absorption maxima. This distortion accompanies the progressive decrease in the extinction coefficient at 328 millimicrons, though the rate of change is not parallel. During the initial stages of oxidation there is a tendency for the colorimetric values to be somewhat less than those based upon the extinction coefficients at 328 millimicrons, and a reversal of this relationship is observed after prolonged oxidation of the vitamin A. Although the absorption maximum for vitamin A alcohol in isopropanol is closer to 325 than to 328 millimicrons, the difference in extinction coefficient between these two wave lengths is small in the case of most fish liver oils.

The importance of conducting the vitamin A determination on the unsaponifiable fraction has been emphasized. It is possible to prepare unsaponifiable extracts without either mechanical or oxidative loss of vitamin A. The ether used for extracting the saponified oil should be redistilled, since ether, even of U. S. P. quality, contains extraneous light-absorbing substances. Experimental data show that the lower vitamin A values obtained when tests are conducted on the unsaponifiable fractions of even high-potency oils are due not only to the removal of irrelevant absorbing substances but in part to the removal of vitamin A oxidation products in the preparation of the unsaponifiable fractions.

The U. S. P. reference cod liver oil No. 2 is shown to be unsuited as a standard for the spectrophotometric and colorimetric

determination of vitamin A. The ultraviolet absorption curve of this oil does not have the shape characteristic of vitamin A; it contains appreciable quantities of irrelevant light-absorbing material not entirely removed by saponification; it is unstable even when stored under nitrogen in the refrigerator for a period of only one month; and there is a marked discrepancy in its vitamin A content estimated by the spectrophotometric and colorimetric methods on either the whole sample or the unsaponifiable fraction.

Acknowledgment

Acknowledgment is made of the technical assistance of Herman Gershon in conducting many of the analyses.

Literature Cited

(1) Adamson, D. G. M., and Evers, N., *Analyst*, 66, 106 (1941).
(2) Baxter, J. G., and Robeson, C. D., *J. Am. Chem. Soc.*, 64, 2411 (1942).
(3) British Pharmacopoeia, p. 579, London, Constable and Co., 1932.
(4) Embree, N. D., IND. ENG. CHEM., ANAL. ED., 13, 144 (1941).
(5) Jamieson, G. S., "Vegetable Fats and Oils", p. 340, New York, Chemical Catalog Co., 1932.
(6) MacWalter, R. J., *Biochem. J.*, 28, 472 (1934).
(7) Marcus, J. K., *J. Biol. Chem.*, 90, 507 (1931).
(8) Morgareidge, K., IND. ENG. CHEM., ANAL. ED., 14, 700 (1942).
(9) Morton, R. A., "Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes", 2nd ed., p. 87, Boston, Jarrell-Ash Co., 1942.
(10) *Ibid.*, p. 96.
(11) Oser, B. L., Melnick, D., and Pader, M., IND. ENG. CHEM., ANAL. ED., 15, 724 (1943).
(12) Pharmacopoeia of U. S., 12th revision, p. 580, Easton, Penna., Mack Printing Co., 1942.
(13) Robinson, F. A., *Biochem. J.*, 32, 807 (1938).
(14) Smith, E. L., *Ibid.*, 33, 201 (1939).
(15) Vitamin Oil Producers' Institute, "Collaborative Study of the Spectrophotometric Test for Vitamin A" (private communication), 1943.
(16) Wilkie, J. B., *J. Assoc. Official Agr. Chem.*, 23, 336 (1940).
(17) Wokes, F., and Willimott, S. G., *Biochem. J.*, 21, 419 (1927).
(18) Woodman, A. G., "Food Analysis", 4th ed., p. 566, New York, McGraw-Hill Book Co., 1942.
(19) Zscheile, F. P., and Henry, R. L., IND. ENG. CHEM., ANAL. ED., 14, 422 (1942).

PRESENTED n part before the joint meeting of the Divisions of Biological and Agricultural and Food Chemistry at the 105th Meeting of the AMERICAN CHEMICAL SOCIETY, Detroit, Mich.

Estimation of Vitamin A in Food Products

BERNARD L. OSER, DANIEL MELNICK, AND MORTON PADER
Food Research Laboratories, Inc., Long Island City, N. Y.

The antimony trichloride method for determining vitamin A in food products has been modified to allow corrections for the presence of inhibitors of the color development, for temperature effects, for variations in the reagent, for turbidities produced in the course of the color development, and for extraneous color present in the final test solution. The unreliability of the direct spectrophotometric method for the assay of foods is demonstrated. The reaction of carotene with antimony trichloride has been critically studied.

THE enrichment of many food products with vitamin A (such as margarine, milk, animal feeds, emergency rations, dietary supplements, etc.) has necessitated the development of a suitable control assay procedure. Spectrophotometric methods for determining vitamin A in such products frequently yield erroneous results, even when the measurements are made on unsaponifiable extracts, or when unfortified blank samples are available for the control tests. The modified spectrophotometric procedure involving destructive irradiation of the vitamin A (*5*, *12*, *14*) to obtain a blank value for irrelevant absorbing materials presumably eliminates the necessity for an unfortified sample. However, this method involves additional analytical steps, the vitamin A is determined by difference, and the blank correction may be very large in relation to the actual vitamin A content.

The Carr-Price colorimetric test for vitamin A (*3*), as modified by Dann and Evelyn (*4*), has been improved to allow corrections for the presence of inhibitors of the color development, for temperature effects, for variations in the reagent, for turbidities produced in the course of the color development, and for extraneous color present in the final test solution. The method has been applied particularly to the determination of preformed vitamin A in food products. The application of the test to fish liver oils (*13*) has proved of value in yielding data to explain various anomalies encountered in spectrophotometric studies.

Reagents

CHLOROFORM, washed three times with an equal volume of water, dried over anhydrous sodium sulfate, distilled, and stored over anhydrous sodium sulfate.

ANTIMONY TRICHLORIDE REAGENT, prepared by dissolving 125 grams of dry antimony trichloride, C. P., in sufficient chloroform to make a final volume of 500 ml. This solution should be filtered if turbid. It is stable at room temperature for several weeks.

VITAMIN A STANDARD, a chloroform solution containing 100 U. S. P. units of vitamin A per ml. This is prepared by dissolving in chloroform a weighed amount of an oil solution of distilled vitamin A esters (Distillation Products, Inc., Rochester, N. Y.) whose vitamin A content is determined spectrophotometrically using the commercial standard conversion factor of 2000 to convert from $E^{1\%}_{1\ cm.}$ 328 mμ to U. S. P. units of vitamin A. The ultraviolet absorption curve of the distilled esters should duplicate that of vitamin *A* (*13*), and the values obtained for vitamin A on the whole oil and unsaponifiable fraction should agree when determined by both the spectrophotometric and colorimetric methods.

0.5 *N* ALCOHOLIC POTASSIUM HYDROXIDE, freshly prepared.

0.5 *N* AQUEOUS POTASSIUM HYDROXIDE.

ETHER, freshly redistilled, discarding the first and last quarters of the distillate.

Apparatus

A direct-reading photoelectric colorimeter, with 620- and 720-millimicron filters. A null-point instrument cannot be used. The direct-reading galvanometer must be critically damped and have a short period. The time required to swing from 0 to 100 and become stable should not be more than 3 seconds. (The Evelyn photoelectric colorimeter, manufactured by the Rubicon Co., Philadelphia, Penna., has proved very satisfactory.)

Interchangeable test tubes for the colorimeter with cork stoppers.

A micropipet, carefully calibrated to deliver 0.1 ml. between two marks.

A 9-ml. automatic pipet (see Figure 1). The opening of the stopcock and the outlet tube should be large enough to permit

complete delivery of the reagent within 1 second. Stopcock lubricants should not be employed.

Analytical Procedure

Preparation of the Unsaponifiable Fraction. A quantity of sample, preferably containing at least 50 U. S. P. units of vitamin A, is saponified by refluxing with the alcoholic potassium hydroxide for 0.5 hour on a water bath. Ten to 15 ml. of alcoholic potassium hydroxide are used for each gram of sample taken. The use of samples larger than 5 grams is not recommended. In the assay of materials low in total solids content it is possible to use a smaller ratio of alcoholic potassium hydroxide to sample. Thus, in the case of enriched milk, 15 ml. of sample are saponified with 45 ml. of the potassium hydroxide solution.

To avoid sampling errors it is desirable, whenever possible, to conduct a preliminary saponification of a large sample, following this by a resaponification of an aliquot using a higher ratio of alcoholic potassium hydroxide to sample. (In margarine, for example, a 10-gram sample is refluxed on a water bath with 45 ml. of alcoholic potassium hydroxide, the suspension is cooled and brought to the 50-ml. mark with the potassium hydroxide solution, and a 20-ml. aliquot is further saponified after the addition of 30 ml. of alcoholic potassium hydroxide.) The solution (or suspension) is allowed to cool, and double its volume of water is added. The resulting solution (or suspension) is then extracted four times with 50 to 100 ml. of ether. The ether extract is washed once with 50 ml. of water, once with 25 ml. of 0.5 *N* aqueous potassium hydroxide, and then with 50-ml. portions of water until the last washing gives no color with phenolphthalein. After drying with anhydrous sodium sulfate it is evaporated to dryness, the last few millimeters being removed at room temperature with a stream of nitrogen. The residue is immediately taken up in sufficient chloroform to give a final concentration of from 5 to 15 U. S. P. units of vitamin A per ml., and the color reaction is then carried out as quickly as possible. A turbid chloroform solution indicates that the ether extract had been insufficiently dried, requiring the addition of anhydrous sodium sulfate to the final test solution.

All operations are performed in amber glassware (*6*).

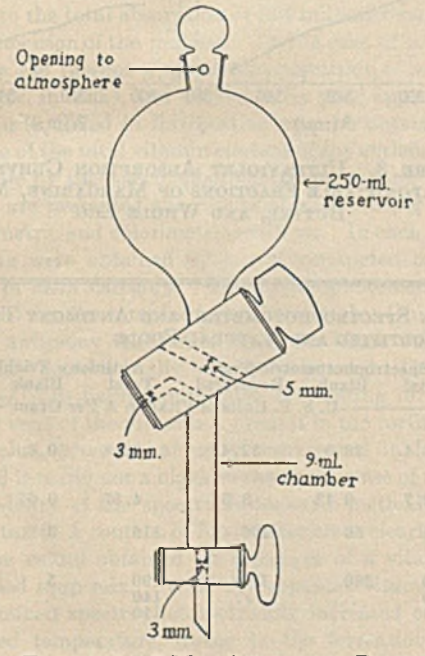


Figure 1. 9-Ml. Automatic Pipet for Delivery of Antimony Trichloride Reagent

Color Development. The center setting of the instrument (using the 620-millimicron filter) is obtained by setting the galvanometer at 100 with 1 ml. of chloroform and 9 ml. of the antimony trichloride reagent. Then to another tube containing 1 ml. of the chloroform test solution are added 9 ml. of the reagent from the automatic pipet. The tube is immediately stoppered and swirled, and the minimal constant galvanometer reading is recorded (*A*) (usually within 4 seconds after the addition of the reagent).

To another tube containing a 1-ml. aliquot of the test solution 0.1 ml. of the vitamin A standard is added from the 0.1-ml. micropipet, followed by 9 ml. of the antimony trichloride reagent, and the minimal constant galvanometer reading is again noted (*B*).

If the test solution is colored, a blank correction is made by measuring the color intensity at 620 millimicrons of a 1 to 10 dilution of the test solution in chloroform (*C*).

To correct for turbidity, occasionally produced during the course of the reaction, the color is developed in a fresh aliquot, and the reading after 4 seconds noted using the 720-millimicron filter (*D*). (The new center setting for the 720-millimicron filter must first be determined as above.)

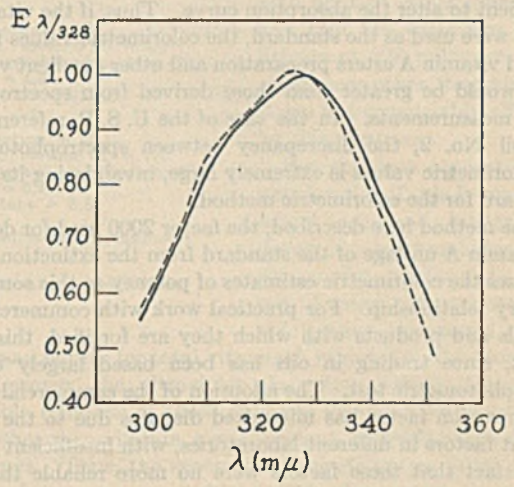


Figure 2. Ultraviolet Absorption Curve of Distilled Vitamin A Esters in Isopropanol

Calculations. Galvanometer readings are converted to photometric densities according to:

$$P.\ D. = 2 - \log G$$

Then,

$$\frac{A - C - D}{1.01\ B - A} \times 10 \times \text{dilution factor} = \text{U. S. P. units of vitamin A per gram of sample}$$

Discussion of Method

Extraneous Color and Turbidity. If the test solution contains pigments that absorb light at 620 millimicrons, the procedure outlined above offers a simple method for correction.

Turbidity, even though not present in the initial test solution, occasionally develops upon the addition of the antimony trichloride reagent. Since the blue color of the vitamin A reaction product shows negligible absorption at 720 millimicrons, the method offers a direct means of measuring and correcting for turbidity. The increment procedure demonstrates that the substances responsible for turbidity do not interfere with the color development.

Selection of the Distilled Vitamin A Esters as the Reference Standard. The ultraviolet absorption curve of this preparation, plotted in Figure 2, is typical of that of vitamin A (see Figure 1 of *13*). At their respective absorption maxima the extinction coefficients of the whole oil and its unsaponifiable fraction show excellent agreement (*13*). The oil solution of the distilled esters is uniform, readily obtainable, and very stable. The extinction coefficient at 328 millimicrons of a stock sample has not changed significantly over a period of 6 months, during which time it was stored under nitrogen in a refrigerator in the absence of light. Even a chloroform solution of the distillate is stable for several days. However, as a precautionary measure, the standard should be freshly prepared each time it is used.

The paper on the spectrophotometric assay of vitamin A (*13*) was concerned in part with experiments on crystalline vitamin A

acetate. This material was selected for that work because it could be considered free of irrelevant absorbing materials and artificial stabilizers. The distilled esters preparation was considered as unsuited since it contains tocopherol. However, in the present study, the presence of tocopherol, imparting greater stability to the oil to be used as the standard, is desirable. The content of tocopherol is insufficient to alter appreciably the absorption curve for vitamin A. Besides, it was suggested by a comparison of the vitamin A values obtained by the spectrophotometric and colorimetric procedures (*13*) that the vitamin A acetate might contain a very small quantity of oxidized vitamin A, insufficient to alter the absorption curve. Thus, if the vitamin A acetate were used as the standard, the colorimetric values for the distilled vitamin A esters preparation and other excellent vitamin A oils would be greater than those derived from spectrophotometric measurements. In the case of the U. S. P. reference cod liver oil No. 2, the discrepancy between spectrophotometric and colorimetric values is extremely large, invalidating its use as a standard for the colorimetric method.

In the method here described, the factor 2000 used for deriving the vitamin A unitage of the standard from the extinction coefficient fixes the colorimetric estimates of potency to this somewhat arbitrary relationship. For practical work with commercial fish liver oils and products with which they are fortified, this is expedient, since trading in oils has been based largely on the spectrophotometric test. The adoption of the commercial standard conversion factor has minimized disputes due to the use of different factors in different laboratories, with insufficient regard for the fact that these factors were no more reliable than the bioassays upon which they were based.

Increment Procedure. The principle of adding to the test solution a known increment of the compound for which assay is being made, rather than basing calculations on a reference curve developed with pure solutions, has been used successfully in several vitamin assay procedures (*1*, *8*, *10*). By adding a known increment of vitamin A to the chloroform test solution the standard is subjected to the same inhibitory (or accelerating) conditions during the color development as the vitamin A originally present. When graded amounts of vitamin A are added as increments, a linear relationship is observed among them on color development, though the intensity of the colors developed may deviate considerably from those obtained in pure solution. Many naturally occurring chemical inhibitors are not of great concern, since they are largely eliminated by assaying the unsaponifiable fraction (*11*). This can be demonstrated in assaying the U. S. P. reference cod liver oil from freshly opened bottles. On the other hand, such variables as the concentration, water and alcohol content, and stability of the reagent, and the influence of temperature on the reaction can best be compensated for by the increment method.

The use of the distilled esters as an increment in the colorimetric assay of unsaponifiable extracts of foods is justified, since the intensity of the color produced by a given quantity of esterified vitamin A reacting with the antimony trichloride reagent is the same as that for an equivalent weight of the free alcohol (*13*).

Stability of Vitamin A in Chloroform Solution. The distilled vitamin A esters solution dissolved in anhydrous, redistilled chloroform to a vitamin A concentration of 100 U. S. P. units per ml. has been found to be stable for as long as 3 days at room temperature (25° C.). Similar tests on a large series of fish liver oils showed no losses of vitamin A during a 24-hour period. However, an oil is occasionally encountered which shows unusual instability. In such cases the antimony trichloride reaction must be carried out on a freshly prepared chloroform solution.

Comparison of the Colorimetric with the Direct Spectrophotometric Method of Assay. Spectrophotometric vitamin A assays can be regarded as reliable only when an unsaponifiable extract of the substance, dissolved in a suitable solvent, gives an ultraviolet absorption curve typical of vitamin A. This is rarely the case in testing foods, as may be seen in Figures 3 and 4. While in every case there was a slight inflection at 325 millimicrons, where vitamin A alcohol absorbs maximally in isopropanol, the curves are atypical of the vitamin. This indicates that other materials than vitamin A were present which absorbed in this region of the spectrum. Thus, extremely high values for

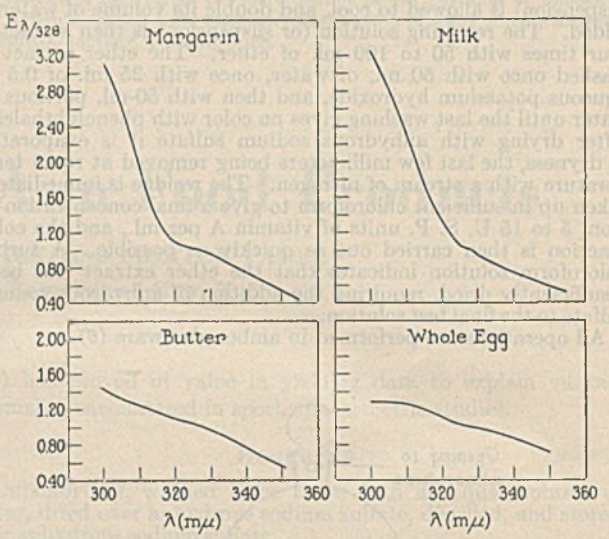


Figure 3. Ultraviolet Absorption Curves of Unsaponifiable Fractions of Margarine, Milk, Butter, and Whole Egg

Table I. Comparison between Spectrophotometric and Antimony Trichloride Tests in Fortified and Natural Foods

Description[a]	Spectrophotometric Test			Antimony Trichloride Test		
	Total	Blank	Recovered	Total	Blank	Recovered
	U. S. P. Units of Vitamin A Per Gram					
Fortified margarine, "15,000 units per lb." (33 units per gram)	70.4	38.0	32.4	31.8	0.8	31.0
Fortified milk, "4000 units per quart" (4.06 units per gram added)	12.7	9.13	3.57	4.57	0.65	3.92
Fortified cracker, "1500 units per cracker"[b] (224 units per gram)	156	50	106	116	3	113
Fortified vitamin-mineral soup mix, "5000 units per ounce" (176 units per gram)	430	260	170	190	5	185
Same, after 2 weeks, 42° C.	570	...	...	140	...	...
Same, after 2 weeks, 55° C.	700	...	...	110	...	...
Fortified chocolate malted milk mix, "3000 units per ounce" (106 units per gram)	142	...	...	108	...	...
Fortified vitamin mineral lozenge, "5000 units per wafer" (517 units per gram)	780	...	...	495	...	...
Poultry feed supplement, 200(?) units per gram	400	...	...	195	...	...
Fresh whole egg, 10 to 20 units per gram (*15*)	24	...	...	24 (+6)[c]	...	...
Butter (80% fat), 35 to 50 units per gram (*15*)	33	...	...	18 (+10)[c]	...	...

[a] Values in quotations represent vitamin A added in preparing commercial sample.
[b] Good agreement between spectrophotometric and colorimetric methods, after correction for apparent vitamin A content of unfortified cracker, indicates that expected value was in error.
[c] As carotene determined by chromatographic adsorption.

Table II. Reliability of Method for Determining Carotene by the Antimony Trichloride Reaction

Test Solution (in 1 ml. of $CHCl_3$)	Photometric Density, 620 Millimicrons: 4 seconds	1.0 minutes	30 minutes	2 hours	3 hours	Comment
10 U. S. P. units of vitamin A (approximately 3 μg.)	0.270	0.226	0.025	0.016	0.016	Typical vitamin A reaction
60 μg. of β-carotene	0.222	0.209	0.268	0.321	0.328	Typical carotene reaction
10 U. S. P. units of vitamin A + 60 μg. of β-carotene	0.502	0.447	0.297	0.323	0.323	Carotene and vitamin A reactions additive

Conclusion: Antimony trichloride reaction can differentiate quantitatively between vitamin A and carotene in pure solutions.

						"Carotene", μg./g.
Unsaponifiable extract of 0.26 gram of whole egg	0.276	0.295	0.166	0.161	0.173	By $SbCl_3$ reaction = 126
Same + 10 U. S. P. units of vitamin A	0.536	0.517	0.188	0.161	0.167	By A. O. A. C. method (2) = 5.0
Same + 60 μg. of β-carotene	0.464	0.506	0.420	0.456	0.468	By chromatographic adsorption (7): Total = 4.5; β-carotene = 2.5
Unsaponifiable extract of 0.38 gram of butter	0.202	0.170	0.042	0.041	0.043	By $SbCl_3$ reaction = 22
Same + 10 U. S. P. units of vitamin A	0.457	0.376	0.059	0.054	0.056	By A. O. A. C. method (2) = 7.1
Same + 60 μg. of β-carotene	0.447	0.403	0.300	0.329	0.332	By chromatographic adsorption (7): Total = 6.4; β-carotene = 5.8

Conclusion: Antimony trichloride reaction on unsaponifiable fraction of above products measures other compounds in addition to carotene and vitamin A.

vitamin A are obtained when the estimates are based on a single spectrophotometric reading at 328 (or 325) millimicrons, even if unsaponifiable extracts are tested.

In the case of fortified preparations the difference between the extinction coefficients at 328 millimicrons of the vitamin A enriched sample and an unfortified, though otherwise identical, blank sample may be used for calculating the amount of vitamin A added. However, this is a difference method, necessitating two separate analyses and the use of a blank sample, which is not always available. Furthermore, the blank value is often very high relative to the total absorption at 328 millimicrons, thus decreasing the precision of the method. In the case of some blank samples there are present appreciable quantities of vitamin A (as, for example, in milk), and while a fairly good approximation of the vitamin A added in fortification may be obtained, a reliable estimate of the total vitamin content of the enriched sample cannot be made by this spectrophotometric procedure.

In Table I are presented the results of assays using both the spectrophotometric and colorimetric methods. In each case confirmatory data were obtained by assays conducted on several preparations in each category. The spectrophotometric blank values obtained for the samples tested were high. On the other hand, in the antimony trichloride tests, the blank values were so low that they could actually have been ignored. Only in the case of milk was this figure appreciable, accounting for approximately 14 per cent of the vitamin A present in the fortified product. This value, however, actually represented in large part vitamin A and is really not a blank in the usual sense of the word.

The unreliability of the spectrophotometric method for estimating the vitamin A content of food materials is clearly demonstrated by the values obtained for a sample of a vitamin- and mineral-fortified soup mix. While the apparent vitamin A content as determined spectrophotometrically increased on storage at an elevated temperature, owing to the formation of nonspecific absorbing substances, the antimony trichloride test showed a decrease in vitamin A content, as was expected. Unfortunately, no stored blank samples were available, but if they had been the spectrophotometric vitamin A values of the incubated samples might have agreed better with the colorimetric figures.

In the case of those samples, which naturally contain inappreciable amounts of vitamin A, the modified spectrophotometric method, involving destructive irradiation of the vitamin A, should theoretically yield the same blank values as those recorded by the direct method for the unfortified samples. Thus, the application of this spectrophotometric technique to the assay of enriched food products, such as those in Table I, must sacrifice precision for specificity. In addition, in any such method it must be shown that the vitamin A is quantitatively converted by the irradiation to materials which fail to absorb light at 328 millimicrons and that the extinction coefficients at 328 millimicrons of substances other than vitamin A are not appreciably affected by this treatment.

For the two natural products listed in Table I—namely, whole egg and butter—the direct spectrophotometric values for preformed vitamin A were not so unreasonable when compared with the bioassay figures for total vitamin A reported in the literature (*15*). However, the ultraviolet absorption curves of the unsaponifiable fractions of these products demonstrated that substances other than vitamin A were present, absorbing light at 325 millimicrons. Thus, the spectrophotometric values must be regarded as being erroneously high. This also casts suspicion on the reliability of the colorimetric value, particularly for whole egg.

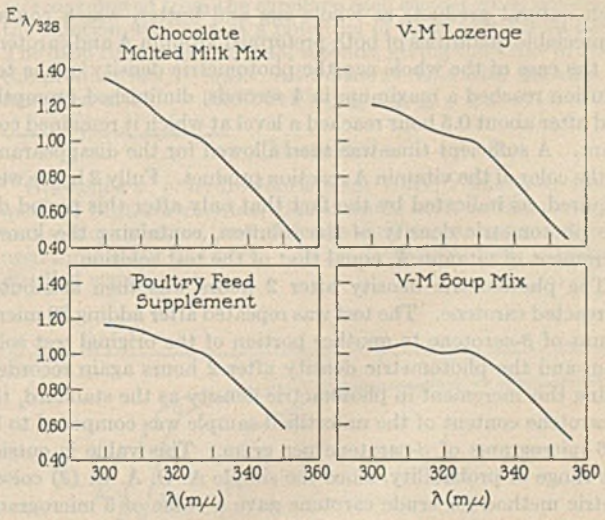


Figure 4. Ultraviolet Absorption Curves of Unsaponifiable Fractions of Chocolate Malted Milk Mix, Vitamin and Mineral Lozenge, Poultry Feed Supplement, and Fortified Soup Mix

The reliability of the spectrophotometric determination of vitamin A in milk was not improved when the saponification was conducted on an ether extract of the milk.

Determination of Preformed Vitamin A and Carotene in the Presence of Each Other. The antimony trichloride test, as applied in the present study, is intended for the measurement of only preformed vitamin A. Carotene also yields a blue color with antimony trichloride (*9*), though of much lower intensity on a weight for weight basis. Furthermore, the color

reaction with carotene differs from the vitamin A reaction in that it attains its maximal intensity only after standing, instead of fading from an almost instantaneous maximum. In Table II the difference between these two reactions is shown.

The maximal color produced by 10 U. S. P. units (about 3 micrograms) of vitamin A and measured at 620 millimicrons is of approximately the same order as that produced by 60 micrograms of β-carotene, although the time at which these maximal intensities are reached differed. Thus, on the basis of equal weights, the ratio of the color intensity of the reaction product of preformed vitamin A to that of β-carotene is approximately 20 to 1; on the basis of biological activity, it is 10 to 1. These data also show that vitamin A and carotene, in solution together, react in an additive manner. In the case illustrated the photometric density at any given time closely approximates the sum of the two individual values. After an interval of 2 hours, when the blue color due to reacted vitamin A has almost completely disappeared, the residual photometric density is a measure of the β-carotene present. Thus, the antimony trichloride reaction can be used to differentiate quantitatively between vitamin A and β-carotene in pure solutions, according to the following equations:

$$(P.\ D.\text{ at 4 seconds}) - \frac{0.222}{0.321} \times (P.\ D.\text{ at 2 hours}) = P.\ D.\text{ due to preformed vitamin A}$$

and

$$(P.\ D.\text{ at 2 hours}) = P.\ D.\text{ due to } \beta\text{-carotene}$$

where

$$P.\ D. = \text{photometric density} = 2 - \log G$$

Attempts were made (see Table II) to apply this method to such natural products as whole egg and butter, which contain appreciable quantities of both preformed vitamin A and carotene. In the case of the whole egg the photometric density of the test solution reached a maximum in 4 seconds, diminished promptly, and after about 0.5 hour reached a level at which it remained constant. A sufficient time was then allowed for the disappearance of the color of the vitamin A reaction product. Fully 2 hours were required, as indicated by the fact that only after this period did the photometric density of the solution, containing the known increment of vitamin A, equal that of the test solution.

The photometric density after 2 hours was then attributed to reacted carotene. The test was repeated after adding 60 micrograms of β-carotene to another portion of the original test solution, and the photometric density after 2 hours again recorded. Using this increment in photometric density as the standard, the β-carotene content of the unfortified sample was computed to be 126 micrograms of β-carotene per gram. This value is outside the range of probability, since the simple A. O. A. C. (*2*) colorimetric method for crude carotene gave a value of 5 micrograms per gram, and the more specific chromatographic adsorption procedure (*7*) showed 4.5 micrograms. In other words, it appears that in whole egg, compounds other than β-carotene are present which give a stable antimony trichloride reaction product absorbing light at 620 millimicrons. (In egg the xanthophylls comprise a large fraction of the carotenoid pigments.) The presence of preformed vitamin A in the unsaponifiable extract prepared from butter was demonstrated by the fact that the initial photometric density was maximal, and promptly decreased to a minimum in 30 minutes, after which it remained constant. However, the estimate of the carotene content from the residual stable photometric density value of the test sample and that produced by a known increment of β-carotene is likewise erroneous; the estimate of 22 micrograms per gram of β-carotene is considerably higher than the value found by the A. O. A. C. colorimetric procedure or by chromatographic adsorption. It is apparent, therefore, that the antimony trichloride method, as described, cannot be used for the determination of carotene in foods.

The use of this reaction for the determination of preformed vitamin A may be subjected to some criticism, since it is assumed that the stable readings obtained after 2 hours are due to compounds which, although not β-carotene, behave like it. When the 2-hour reading is large relative to that noted at the end of 4 seconds (58 per cent in the case of the whole egg), errors due to the above assumption may be appreciable, and may therefore invalidate the test. When the stable reading is small relative to that at 4 seconds (20 per cent in the case of butter), a more reliable value for preformed vitamin A is obtained.

In the authors' opinion, preformed vitamin A in food materials should be estimated by the improved antimony trichloride reaction and carotene should be estimated independently, preferably by chromatographic adsorption. A small correction may then be applied for the reaction of the carotenoids with the antimony trichloride reagent in the test for vitamin A. It is apparent, from the results shown in Table II, that in testing a product in which as much as half of the vitamin A potency is due to β-carotene, the amount of color at 4 seconds due to the reaction of carotene is small, of the order of 8 per cent.

The simple procedure, suggested by Dann and Evelyn (*4*), of measuring the yellow color of the chloroform solution prior to its reaction with the antimony trichloride reagent and attributing this to carotene, has been found to yield "carotene" values for a number of food materials as much as six times greater than those obtained by the simple A. O. A. C. (*2*) colorimetric procedure, which is admitted to be not specific for the biologically active carotenoids (*7*).

Summary

A modification of the antimony trichloride method is described for determining vitamin A in food products. The method compensates for the effect of inhibitors of the color reaction, variations in the reagent, temperature effects, and extraneous colors and turbidity that sometimes interfere.

When foods are assayed by the spectrophotometric procedure, erroneously high estimates are usually obtained despite preliminary extraction of their unsaponifiable fractions for the tests. In such cases the absorption curves deviate markedly from the characteristic vitamin A curve. The use of unfortified blank samples (if they are available) increases the specificity of this method with the possible sacrifice of precision. The modified spectrophotometric procedure involving ultraviolet destruction of vitamin A is a "difference method", the blank corrections being large relative to the true vitamin A values. In the colorimetric method a blank sample is usually unnecessary because of the greater specificity of the procedure for determining vitamin A in foods.

β-Carotene reacts with the antimony trichloride reagent to give a stable blue color. Determination of both carotene and preformed vitamin A, in the presence of each other in pure solutions, is possible by this reaction. However, in natural foods, substances other than carotene yield blue reaction products, invalidating use of this test for the determination of carotene. The amount of color due to the reaction of pure β-carotene with the reagent is very much less than that due to preformed vitamin A, expressed either on the basis of equal weight or equivalent biological activity. Thus, only a minimal correction for β-carotene, if any at all, need be applied to colorimetric preformed vitamin A values in the assay of foods containing both forms of the vitamin. Carotene should be estimated independently, preferably by chromatographic adsorption.

Acknowledgment

The authors wish to acknowledge the technical assistance of Milton Wolf in the course of this investigation.

Literature Cited

(1) Andrews, J. S., *Cereal Chem.*, 20, 3 (1943).
(2) Assoc. Official Agr. Chem., "Official and Tentative Methods of Analysis", 5th ed., p. 369, 1940.
(3) Carr, F. H., and Price, E. A., *Biochem. J.*, 20, 497 (1926).
(4) Dann, W. J., and Evelyn, K. A., *Ibid.*, 32, 1008 (1938).
(5) Demarest, B., *Z. Vitaminforsch.*, 9, 20 (1939).
(6) Embree, N. D., IND. ENG. CHEM., ANAL. ED., 13, 144 (1941).
(7) Fraps, G. S., and Kemmerer, A. R., *Ibid.*, 13, 806 (1941).
(8) Hodson, A. Z., and Norris, L. C., *J. Biol. Chem.*, 131, 621 (1939).
(9) Kuhn, R., and Brockmann, H., *Z. physiol. Chem.*, 206, 41 (1931).
(10) Melnick, D., *Cereal Chem.*, 19, 553 (1942).
(11) Morton, R. A., "Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes", 2nd ed., p. 87, Boston, Jarrel-Ash Co., 1942.
(12) Neal, R. H., Haurand, C. H., and Luckmann, A. H., IND. ENG. CHEM., ANAL. ED., 13, 150 (1941).
(13) Oser, B. L., Melnick, D., and Pader, M., *Ibid.*, 15, 717 (1943).
(14) Peacock, P. R., *Lancet*, 11, 328 (1926).
(15) Sherman, H. C., "Chemistry of Food and Nutrition", 6th ed., pp. 569, 570, New York, Macmillan Co., 1941.

PRESENTED in part before the joint meeting of the Divisions of Biological and Agricultural and Food Chemistry at the 105th Meeting of the AMERICAN CHEMICAL SOCIETY, Detroit, Mich.

Polarographic Analysis of Dilute Solutions of Bismuth

DONALD F. SWINEHART[1], A. B. GARRETT, AND WILLIAM M. MACNEVIN
The Ohio State University, Columbus, Ohio

Data are presented for the quantitative polarographic analysis of dilute solutions of bismuth in the form of the complex tartrate in acetate-buffered solutions. Such solutions (10^{-5} to 3×10^{-3} molal) can be analyzed with an average error of 1 mg. of bismuth per liter. The diffusion current is not quite a linear function of the bismuth concentration from 1×10^{-5} to 3×10^{-3} molar solutions at the pH value and tartrate concentration investigated in this work. The half-wave potential is a function of the pH of the solution.

IN THE course of an investigation of the ionic equilibria of bismuth it became necessary to analyze dilute solutions of bismuth (in the range 10^{-2} to 10^{-5} molal). This paper presents data on the use of the polarographic method for the analysis of such solutions.

Early attempts were made to obtain polarograms for the reduction of bismuth in hydrochloric acid using a Heyrovský micropolarograph[2]. At these high hydrogen-ion concentrations the half-wave potential was so close to zero applied potential that little or no part of the residual current curve was obtained to be used in evaluating the diffusion currents. In addition, the curves exhibited erratic maxima. However, the half-wave potential of a bismuth-tartrate complex ion is known to be more negative than that of Bi^{+++} by several tenths of a volt (*6*). Such a system gave promise of avoiding the difficulty experienced with the hydrochloric acid solutions; a brief study of this system is reported in this paper.

Apparatus

A manual apparatus, similar to that of Lingane and Kolthoff (*3*), was used for this work. A 9999-ohm decade resistance box was connected in series with the cell consisting of the dropping mercury electrode and a normal calomel half-cell. The drop in potential across this resistance was measured with a student potentiometer (±0.0001 volt). The currents flowing were calculated from Ohm's law. Another student potentiometer was used as a volt-box for tapping off known potentials (to be impressed across the cell) from Edison cells. A galvanometer (2×10^{-8} ampere per mm. sensitivity) was used for null-point readings only. The dropping mercury electrode was attached to a leveling bulb and manometer (*3*). No rubber was in contact with the mercury. With a head of 35.0 cm. of mercury, an average of 1.443 mg. of mercury flowed from the capillary each second when immersed in the solution used and at a potential of 0.500 volt across the cell. The drop time averaged 3.43$_5$ seconds, giving the value of 1.57 for the product $m^{2/3}t^{1/6}$ (*2*). All work was done with the cells thermostated at 35° ± 0.05° C.

Materials

Grasselli's C. P. hydrochloric acid, Baker's analyzed (reagent quality) bismuth chloride, glacial acetic acid, sodium hydroxide, and sodium acetate, and Coleman-Bell's C. P. tartaric acid were used throughout this work. All solutions were made with double-distilled water.

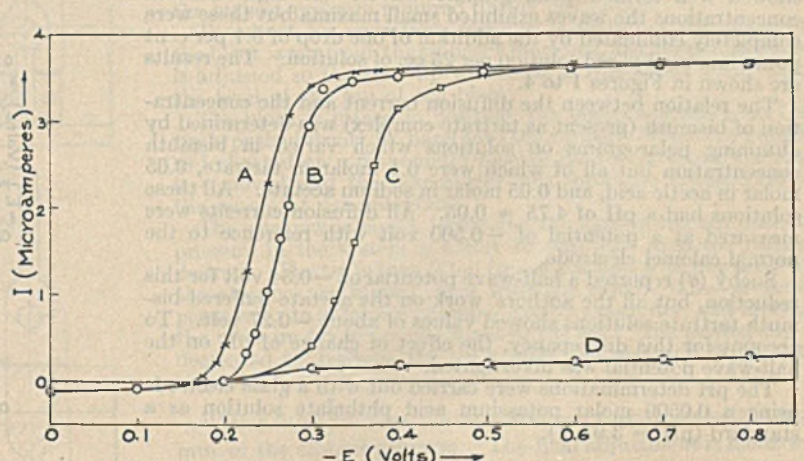


FIGURE 1. TYPICAL POLAROGRAMS
A. 100 mg. of bismuth per liter, pH 4.20
B. 100 mg. of bismuth per liter, pH 4.75
C. 100 mg. of bismuth per liter, pH 5.90
D. 0 mg. of bismuth per liter, pH 4.75
Each solution was 0.1 molar in tartrate and buffered with acetic acid-sodium acetate mixtures

[1] Present address, Eastman Kodak Co., Rochester, N. Y.

[2] Page and Robinson (*4*) have recently published an account of the polarographic analysis of bismuth in hydrochloric and sulfuric acid solutions. Most of the authors' work was done before this paper was published.

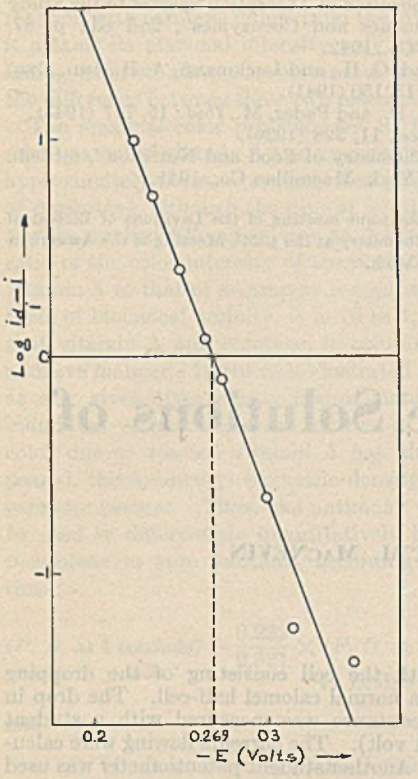


FIGURE 2. PLOT USED TO FIND VALUE OF HALF-WAVE POTENTIAL OF *B*, FIGURE 1

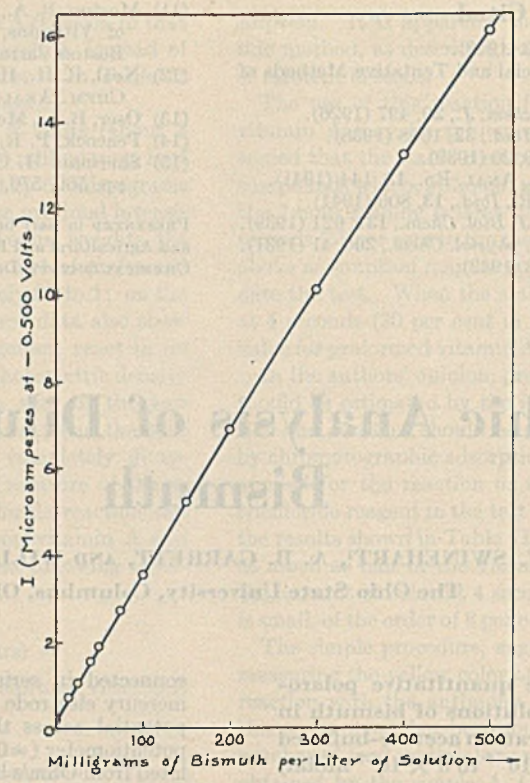


FIGURE 3. VARIATION OF MEASURED CURRENT WITH CHANGE OF BISMUTH CONCENTRATION

All solutions were 0.1 molar in tartrate, 0.05 molar in acetic acid, and 0.05 molar in sodium chloride. pH 4.75 ± 0.05

solutionscontaining the supporting electrolyte only—i. e., tartrate and acetate buffer system (*cf.* Figure 1, *D*)—from the measured currents.

The half-wave potentials were obtained from a plot of E *vs.* $\log \frac{i_d - i}{i}$ where $E = E_{1/2}$ when $\log \frac{i_d - i}{i} = 0$. This plot for curve *B*, Figure 1, is shown in Figure 2.

Currents (measured as total current at −0.500 volt) at various bismuth concentrations are shown graphically in Figure 3. The curve is strictly linear below a bismuth concentration of 100 mg. per liter. The ratio of i_d (microamperes) to concentration of bismuth (mg. per liter) has an average value of 0.0340 in this range. Above a bismuth concentration of 100 mg. per liter the curve deviates slightly from linearity. At a bismuth

Experimental

A standard solution of bismuth trichloride in hydrochloric acid was prepared. It was standardized gravimetrically by removing the chloride ions by twice evaporating the solution nearly to dryness with excess nitric acid, precipitating the bismuth as the basic carbonate, and igniting the basic carbonate to the oxide which was weighed. All other solutions of bismuth were prepared by dilution of this standard solution in calibrated flasks.

Atmospheric oxygen was removed from all samples by bubbling purified nitrogen through them for 10 minutes.

Dilute bismuth solutions, 0.1 molar in tartrate and buffered with acetic acid–sodium acetate mixtures, were investigated; they showed well formed polarographic waves. At high bismuth concentrations the waves exhibited small maxima but these were completely eliminated by the addition of one drop of 0.1 per cent potassium methyl red solution per 25 cc. of solution. The results are shown in Figures 1 to 4.

The relation between the diffusion current and the concentration of bismuth (present as tartrate complex) was determined by obtaining polarograms on solutions which varied in bismuth concentration but all of which were 0.1 molar in tartrate, 0.05 molar in acetic acid, and 0.05 molar in sodium acetate. All these solutions had a pH of 4.75 ± 0.05. All diffusion currents were measured at a potential of −0.500 volt with reference to the normal calomel electrode.

Suchý (*6*) reported a half-wave potential of −0.34 volt for this reduction, but all the authors' work on the acetate-buffered bismuth tartrate solutions showed values of about −0.27 volt. To account for this discrepancy, the effect of change of pH on the half-wave potential was investigated.

The pH determinations were carried out with a glass electrode using a 0.0500 molar potassium acid phthalate solution as a standard (pH = 3.97, *1*).

Data and Discussion

Typical data for complete polarograms of the bismuth-tartrate complex are shown graphically in Figure 1 where *I* is the measured current in microamperes.

Diffusion currents, i, and limiting diffusion currents, i_d, were obtained by subtracting the residual currents, obtained with

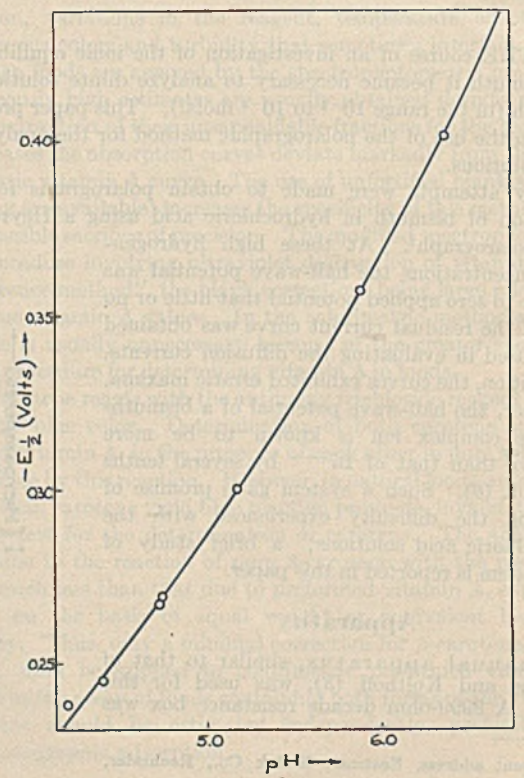


FIGURE 4. HALF-WAVE POTENTIAL AS A FUNCTION OF pH

For solutions containing 100 mg. of bismuth and 0.1 mole of tartrate per liter

concentration of 300 mg. per liter the above ratio drops to 0.0330, at 500 mg. per liter to 0.0318, and at 600 mg. per liter to 0.0308.

It was found that high concentrations of sodium chloride lowered the slope of the curve in Figure 3 proportionately to the sodium chloride concentration. One molar sodium chloride lowered the slope from 0.0340 to 0.0318, and 2 and 3 molar sodium chloride lowered it to 0.0296 and 0.0276, respectively. This effect has been noted by Peracchio and Meloche (*5*).

The polarographic data, plotted on a large scale, were used for the analysis of dilute solutions of bismuth. An extrapolation of this plot (Figure 3) to zero concentration gave an accurate value of the residual current of 0.20 microampere at −0.500 volt. Values of the diffusion currents were reproducible to an equivalent of about 1 mg. of bismuth per liter at all concentrations.

The different values of $E_{1/2}$ (reported by Suchý, *6*, and that above) are explained by the fact that $E_{1/2}$ is shifted greatly by change of pH of the solutions. Figure 4 shows a plot of $E_{1/2}$ versus pH for solutions having 100 mg. of bismuth per liter. The value of the diffusion current is not affected by change of pH, if pH < 7, within the limits of experimental error. Changes of pH below 7 only translate the wave along the E axis, Figure 1. For pH > 7 the current voltage curves showed two waves suggesting a stepwise reduction of bismuth but no definite mechanism is proposed to interpret these two waves.

The shift of $E_{1/2}$ to greater negative potentials as the pH values increase corresponds to the well known fact that metal-tartrate complex ions in general are more stable in alkaline than in acid media.

Acknowledgment

The authors wish to express their appreciation to E. I. du Pont de Nemours & Company for the fellowship which made this work possible; this represents an excerpt of the research work done with the aid of that fellowhsip.

Literature Cited

(1) Clark, "Determination of Hydrogen Ions", p. 485, Baltimore, Williams & Wilkins Co., 1928.
(2) Kolthoff and Lingane, "Polarography", p. 60, New York, Interscience Publishers, 1941.
(3) Lingane and Kolthoff, *J. Am. Chem. Soc.*, 61, 825–34 (1939).
(4) Page and Robinson, *J. Soc. Chem. Ind.*, 61, 93 (1942).
(5) Peracchio and Meloche, *J. Am. Chem. Soc.*, 60, 1770 (1938).
(6) Suchý, *Collection Czechoslov. Chem. Commun.*, 3, 354–66 (1931).

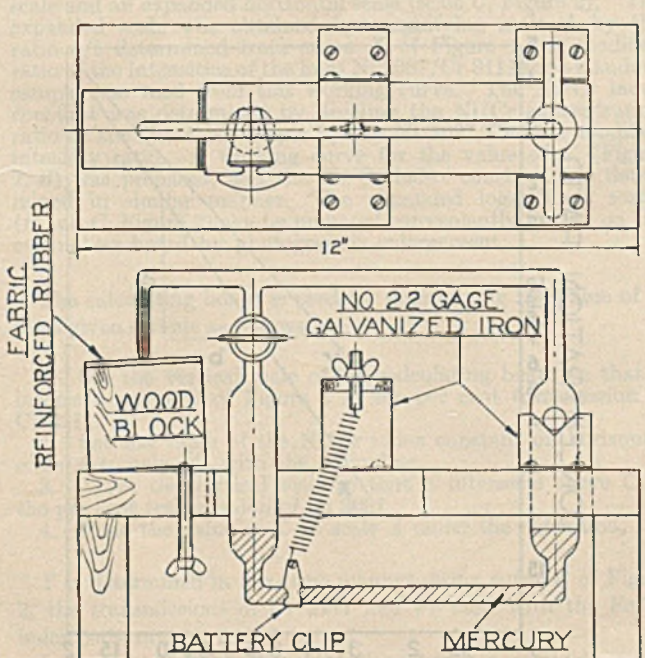
A Modified Bailey Pressure Regulator

GEORGE A. DALIN

Plax Corporation, Hartford, Conn.

BAILEY (*1*) recently described a manostat which, under certain circumstances, was inclined to "bounce", and explained how the bouncing could be eliminated by grinding a bevel on the tip of the capillary leak.

A duplicate was constructed by the author, but it was found that the bevel caused a poor seal between the tip and the rubber seat and prevented attaining a vacuum lower than about 40 mm. in the apparatus. Furthermore, bouncing set in when attempt was made to set the pressure at 80 mm. Several tips were constructed with various bevels and tried with rubber seats of various hardnesses. As was to be expected, increasing the inside diameter of the tip and the angle of the bevel increased the average pressure at which the manostat functioned smoothly. Each tip covered a range of about 30 mm.



MANOSTAT

The modification shown in the diagram is, in effect, a variable bevel. The capillary is first drawn down to a very fine tip and then ground down on a steel plate, using coarse emery and kerosene, until the opening is of such a size that when unobstructed and connected into the apparatus, the pump will take the pressure down to a value which is about 20 mm. higher than the highest pressure required during distillation.

To adjust for operation, mercury is placed in the manostat, the block is removed, the stopcock is opened, and the spring tension is adjusted so that the tip will come to rest about 1 cm. below the level of the top of the block.

The block is placed in position, the pump is started, and the tip is pushed firmly by hand into the rubber. When the pressure as indicated on a manometer reaches its lowest value, the hand is removed and the manometer will show the lowest pressure the manostat can maintain.

The setscrew is raised, increasing the leak at the tip until the pressure in the system is about 10 mm. less than the pressure desired. The tip is then lifted free of the seat with one hand and the stopcock is flipped over with the other. This procedure permits air to enter the chamber over the mercury, and increases the pressure for which the manostat is set. The setting can be decreased by pushing the tip against the seat and flipping the stopcock.

With a little practice, the manostat can thus be set within 2 to 3 mm. of the desired pressure. The final adjustment is made with the setscrew and an accuracy of 0.5 mm. in the setting is easily attained. Any drift due to fatigue of the rubber or dirt particles can be quickly neutralized by means of the setscrew.

Literature Cited

(1) Bailey, A. J., IND. ENG. CHEM., ANAL. ED., 15, 283 (1943).

Spectrographic Determination of Nickel and Chromium in Stainless Steel

J. HORACE COULLIETTE, American Cast Iron Pipe Company, Birmingham, Ala.

Nickel and chromium in stainless steel can be determined by the spectrographic method, using the interrupted spark as a source of radiation. The method is derived from the conventional "internal standard" procedure, and does not require the presence of a "standard" element in a fixed percentage. Details of procedure for preparing working curves and making determinations are given. The method is recommended for control of routine melting operations. Its precision compares satisfactorily with ordinary analytical determinations.

THE internal standard method of spectrographic analysis as commonly used is dependent upon the assumption that the concentration of the element used as a standard does not vary from one sample to another. It is frequently desirable to analyze materials consisting of three component elements present in various percentages. A typical material of this type is stainless steel in which the principal constituents are nickel, chromium, and iron. Typical analyses show that elements present in minor amounts, such as silicon, carbon, manganese, sulfur, etc., will constitute approximately 2 per cent of the material, as manufactured under average conditions. Therefore, the relation of the principal components of a stainless steel may be represented by the equation

$$\%\,\mathrm{Ni} + \%\,\mathrm{Cr} + \%\,\mathrm{Fe} = 98 \qquad (1)$$

The ratio of the concentration of nickel to the concentration of chromium may be represented by X, and the ratio of the concentration of iron to the concentration of chromium may be designated by Y. Then if quantities X and Y can be experimentally determined, the concentrations of the elements can be determined by the solution of the algebraic equations:

$$\mathrm{Ni} + \mathrm{Fe} + \mathrm{Cr} = 98 \qquad (1)$$
$$\mathrm{Ni} - X\mathrm{Cr} = 0 \qquad (2)$$
$$\mathrm{Fe} - Y\mathrm{Cr} = 0 \qquad (3)$$

$$\mathrm{Cr} = \frac{98}{1 + X + Y} \qquad (4)$$

and

$$\mathrm{Ni} = X\mathrm{Cr} \qquad (2)$$

A spectrographic method for determining concentration ratios X and Y has been developed and applied with satisfactory results for the control of the melting of stainless steel in the electric furnace.

A series of five samples of stainless steel was prepared by melting in an induction furnace and casting small rods in a cast-iron mold. The rods were 5 cm. (2 inches) long and 0.6 cm. (0.25 inch) in diameter. Casting in the iron mold caused very rapid freezing of the sample, so that segregation at the grain boundaries was reduced to a minimum. The rods were ground so that the ends were conical, having an included angle of 140°. They were aligned axially in electrode holders to form a 2-mm. spark gap.

The excitation energy was supplied by a commercial type (A. R. L., Dietert) high-voltage spark circuit including a synchronous interrupter. The circuit was adjusted to contain inductance of 0.04 millihenry and capacitance of 0.007 microfarad. The power input was ⅔ kva. According to the manufacturer, the peak voltage across the condenser is 35,000 volts. The sample was sparked for 30 seconds before beginning the exposure. The presparking time and the sparking conditions must be accurately duplicated in order to obtain satisfactory reproducibility.

The spectrograph used was a Baird (*1*) 3-meter grating instrument, and the spectrum was photographed in the second order. The exposure time for Eastman Spectrum Analysis No. 1 film was of the order of 10 seconds. The film processing was performed in an accurately reproducible manner (*4*) in solutions held at a thermostatically controlled temperature of 70° F.

The series of five samples of stainless steel was carefully analyzed by chemical methods (Table I). The spectra of these five samples were studied and three lines were chosen for measurement. The lines used for the measurements described in this paper are listed in Table II with the data given in the M. I. T. Wavelength Tables (*2*). Later work has shown that these lines are subject to interference when copper, molybdenum, or titanium is present; hence a more suitable group of lines is Ni 3087.077, Cr 3169.192, and Fe 3259.048.

Since the densitometer used in this laboratory indicates percentage transmission, it is convenient to prepare calibration curves in terms of this quantity.

TABLE I. CHEMICAL ANALYSIS OF STANDARD SAMPLES

Sample No.	1	2	3	4	5
Nickel, %	7.72	14.1	17.5	26.2	35.7
Chromium, %	20.2	28.6	28.5	17.6	19.4
X = Ni/Cr	0.382	0.493	0.614	1.49	1.84
Y = Fe/Cr	3.42	1.90	1.79	3.04	2.06

TABLE II. SPECTRUM LINES SELECTED FOR MEASUREMENT

Wave Length	Element	Intensity Arc	Intensity Spark
2997.301	Fe II	...	60
3087.077	Ni II	...	150
3111.941	Cr II	...	40

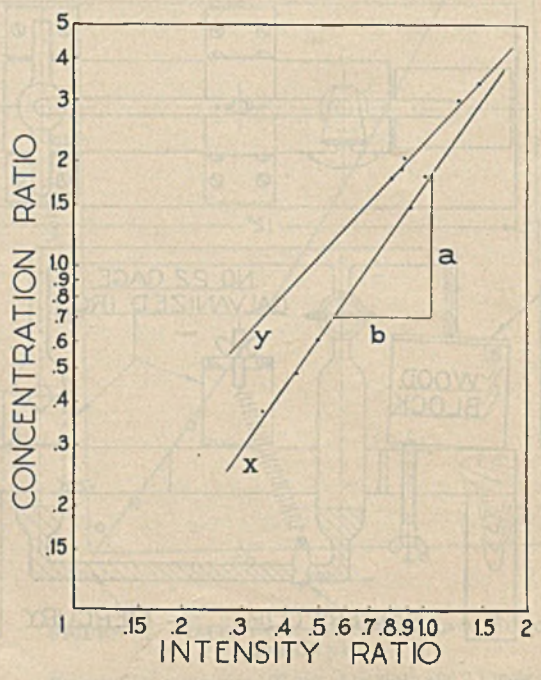


FIGURE 1

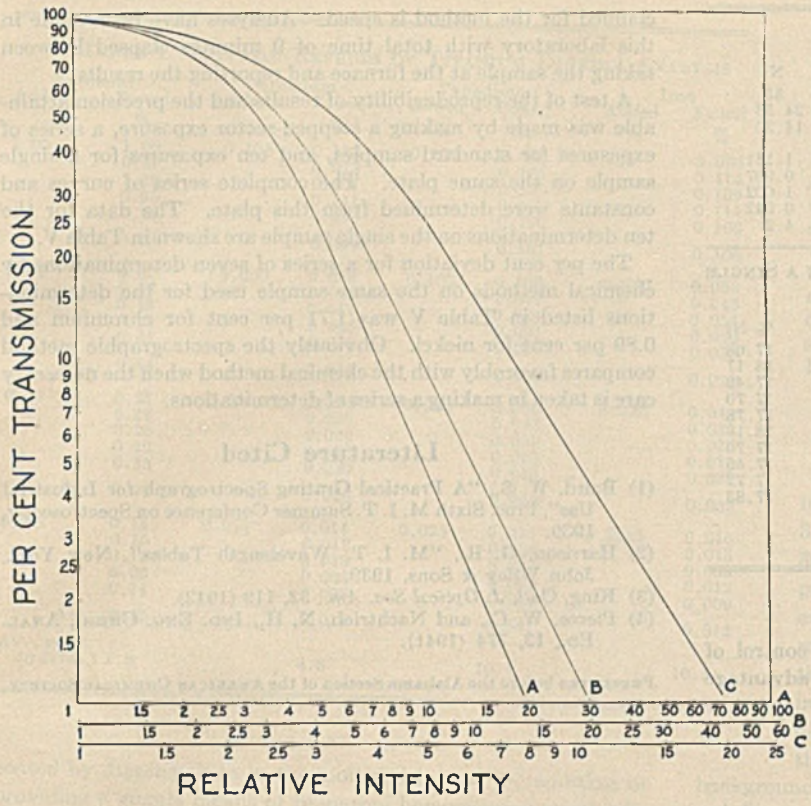


FIGURE 2

The log T/log I curve (Figure 2, A), for the film emulsion was determined by using a step sector, and was plotted on a calculating board as suggested by King (*3*). The spectra of the five samples were measured and the intensity ratios of the lines Ni 3087/Cr 3111 and Fe 2997/Cr 3111 for each sample were determined from the log T/log I curve. The logarithms of the intensity ratios were plotted against the logarithms of concentration ratios X and Y (Figure 1).

The slopes (a/b) of the curves in Figure 1 were determined. A working curve for the value of X (Figure 2, C) was prepared by plotting the data of the log T/log I curve on the same vertical scale and an expanded horizontal scale (scale C, Figure 2). The expanded scale was obtained by magnifying scale A by the ratio a/b determined from curve X of Figure 1. A modified ratio of the intensities of the lines Ni 3087/Cr 3111 for a standard sample was read from this working curve. The Ni/Cr index constant was determined by dividing the Ni/Cr concentration ratio of the standard sample by the Ni 3087/Cr 3111 modified intensity ratio. A working curve for the value of Y (Figure 2, B) was prepared, and the Fe/Cr index constant was determined in similar manner. The expanded logarithmic scales (B and C, Figure 2) can be prepared conveniently for use on the calculating board by photographic enlargement.

The calculating board is used for determining the value of X for a given sample as follows:

1. Set the vertical scale of the calculating board so that it intersects curve C of Figure 2 at the per cent transmission of Cr 3111.
2. Set the value of the Ni/Cr index constant on horizontal scale A to coincide with the index line.
3. Move the vertical scale so that it intersects curve C at the per cent transmission of Ni 3087.
4. Read the value of X on scale A under the index line.

Y is determined in the same manner, using curve B of Figure 2, the transmissions of Cr 3111 and Fe 2997 with the Fe/Cr index constant.

In order to obtain the greatest accuracy a complete set of curves should be prepared for each plate or film, but this is not feasible in high-speed routine work. Since the log T/log I curve is determined by the characteristics of the emulsion and the developing procedure, the use of accurate time and temperature control makes it necessary to redetermine this curve only when a different emulsion or a new batch of developer is put in service.

The concentration ratio *vs.* intensity ratio curves are determined by the type of material, the sample, and the excitation procedure. Hence, it should be necessary to redetermine these curves only when changes are made in one or more of these factors. The desirability of exact repetition of the excitation procedure with particular regard to voltage stabilization and timing should be emphasized.

The routine of analysis consists of five steps: (1) preparation of sample, (2) exposure and processing of spectrogram, (3) measuring per cent transmissions of three spectral lines, (4) reading concentration ratios X and Y from the working curves, and (5) slide-rule computation of the concentrations from Equations 2 and 4.

In order to study the reliability of the method in routine analysis, a number of heats were analyzed by both chemical and spectrographic methods (Table III). The agreement between chemical and spectrographic values is indicated by expressing the spectrographic value as a fraction of the chemical value. The standard deviations of the two series of ratios are shown in Table IV. The per cent deviations in Table IV are due to the combined errors of the chemical and spectrographic procedures; all were routine determinations in which no special effort was made to attain high accuracy.

TABLE III. COMPARISON OF ROUTINE CHEMICAL AND SPECTROGRAPHIC DETERMINATIONS

Heat No.	Chromium			Nickel		
	Spectrographic	Chemical	Ratio of spectrographic to chemical	Spectrographic	Chemical	Ratio of spectrographic to chemical
2170	29.89	28.70	1.042	15.37	15.42	0.997
2173	29.62	26.48	1.118	15.72	16.20	0.970
2190	30.68	27.97	1.096	15.63	16.08	0.972
2193	29.78	28.56	1.033	15.78	15.87	0.994
2197	29.30	27.78	1.055	15.56	15.72	0.990
2703	28.85	28.97	0.996	14.82	15.17	0.977
2725	28.70	28.12	1.020	15.23	14.38	1.059
2728	28.91	27.99	1.033	15.02	15.80	0.951
2731	30.52	29.18	0.955	15.00	15.80	0.949
2740	30.00	29.46	1.018	14.58	15.09	0.966
2743	28.92	28.23	1.024	15.18	15.01	1.011
2746	31.56	29.17	1.083	14.92	14.66	1.018
2750	28.50	27.21	1.047	14.81	14.50	1.021
2792	30.80	29.31	1.050	15.40	15.16	1.016
2795	32.00	30.82	1.037	16.32	16.28	1.002
2797	32.21	31.67	1.017	15.75	16.50	0.955
2800	29.79	30.14	0.988	16.37	14.47	1.131
2816	30.37	29.60	1.026	15.06	14.38	1.047
2819	29.59	30.47	0.972	14.26	15.41	0.925
2821	31.12	30.23	1.029	16.10	15.41	1.045
2822	30.31	28.84	1.051	14.91	16.44	0.907
2825	28.74	28.40	1.012	20.69	20.23	1.023
2827	30.57	29.12	1.048	20.65	20.23	1.021
2829	29.75	28.57	1.042	20.90	20.90	1.000
2832	30.80	29.20	1.054	20.95	20.90	1.002
2835	28.13	29.72	0.947	19.19	19.48	0.985
2844	27.72	27.39	1.013	24.53	23.50	1.044
2847	28.88	28.01	1.031	20.07	20.03	1.002
2851	30.35	30.14	1.007	20.55	19.96	1.030
2854	30.77	29.38	1.047	21.08	20.44	1.031
2865	30.21	29.23	1.033	21.05	21.00	1.002
2868	28.47	27.90	1.019	24.08	22.62	1.065
2871	29.75	28.26	1.052	20.97	21.00	0.999
2874	30.63	29.20	1.049	20.97	21.20	0.989
2884	31.65	29.51	1.072	21.68	22.57	0.961

TABLE IV. STANDARD DEVIATIONS

	Cr	Ni
No. of samples	35	35
Maximum % concentration	32.21	24.53
Minimum % concentration	27.72	14.26
Spectrographic/chemical ratio		
Maximum	1.118	1.131
Minimum	0.947	0.907
Average	1.032	1.002
Standard deviation	0.034	0.042
% deviation	3.3	4.2

TABLE V. PRECISION AND REPRODUCIBILITY TEST ON A SINGLE SAMPLE

Per Cent Transmission						
Cr 3169	Ni 3087	Fe 3259	X	Y	% Cr	% Ni
14.6	19.6	49.2	1.835	0.624	28.35	17.68
15.5	22.1	51.3	1.770	0.631	28.80	18.17
12.4	17.2	44.5	1.796	0.605	28.80	17.42
13.9	19.2	48.0	1.790	0.616	28.75	17.70
15.4	22.1	52.7	1.760	0.612	29.05	17.78
16.4	22.6	53.6	1.790	0.635	28.59	18.16
15.0	21.0	51.3	1.793	0.616	28.75	17.70
14.0	19.8	50.0	1.778	0.602	28.99	17.45
17.8	24.4	57.7	1.813	0.621	28.52	17.72
17.4	23.6	56.0	1.835	0.629	28.38	17.83
		σ Cr = 0.223		% deviation = 0.77		
		σ Ni = 0.237		% deviation = 1.33		

Conclusions

The precision of the method is sufficient for the control of melting operations of stainless steel. The primary advantage claimed for the method is speed. Analyses have been made in this laboratory with total time of 9 minutes elapsed between taking the sample at the furnace and reporting the results.

A test of the reproducibility of results and the precision attainable was made by making a stepped-sector exposure, a series of exposures for standard samples, and ten exposures for a single sample on the same plate. The complete series of curves and constants were determined from this plate. The data for the ten determinations on the single sample are shown in Table V.

The per cent deviation for a series of seven determinations by chemical methods on the same sample used for the determinations listed in Table V was 1.71 per cent for chromium and 0.89 per cent for nickel. Obviously the spectrographic method compares favorably with the chemical method when the necessary care is taken in making a series of determinations.

Literature Cited

(1) Baird, W. S., "A Practical Grating Spectrograph for Industrial Use", Proc. Sixth M. I. T. Summer Conference on Spectroscopy, 1939.
(2) Harrison, G. R., "M. I. T. Wavelength Tables", New York, John Wiley & Sons, 1939.
(3) King, Carl, *J. Optical Soc. Am.*, 32, 112 (1942).
(4) Pierce, W. C., and Nachtrieb, N. H., IND. ENG. CHEM., ANAL. ED., 13, 774 (1941).

PRESENTED before the Alabama Section of the AMERICAN CHEMICAL SOCIETY.

A Fused Salt Technique in Spectrochemical Analysis

N. H. NACHTRIEB[1], D. H. JOHNSON, AND K. S. DRESS
Pittsburgh Plate Glass Co., Barberton, Ohio

FREQUENTLY the chemical spectroscopist is called upon to analyze powdered substances for minor constituents. Broadly speaking, analyses of material in the solid state present more difficulties than determinations of constituents in solution. Substances which are soluble in water, acids, or other common solvents entail few problems; in such cases it is usually feasible to evaporate a drop of a solution of the material, containing an added internal standard when necessary, on the surfaces of smooth-faced graphite electrodes and to excite the residue in a high-voltage alternating current arc (*2*).

Substances which do not dissolve in the common solvents are generally treated according to the time-honored practice of vaporizing and exciting them from the cored anode of a direct current arc. As is well known, analyses carried out by means of the direct current arc often fall short of the accuracy which is possible in spectrochemical analysis. Not all of the blame can be laid to the intrinsic properties of the direct current arc, however. A common source of error, both in the preparation of standard powder samples and in the analysis of unknown samples, is lack of homogeneity. It is an exceedingly arduous task to disperse small amounts of added material uniformly throughout a matrix in the preparation of standard powders.

Confronted with this problem in the analysis of titanium dioxide, the authors have developed a method in which samples are fused with potassium bisulfate, and graphite electrodes are

[1] Present address, University of Chicago Metallurgical Laboratory, Chicago, Ill.

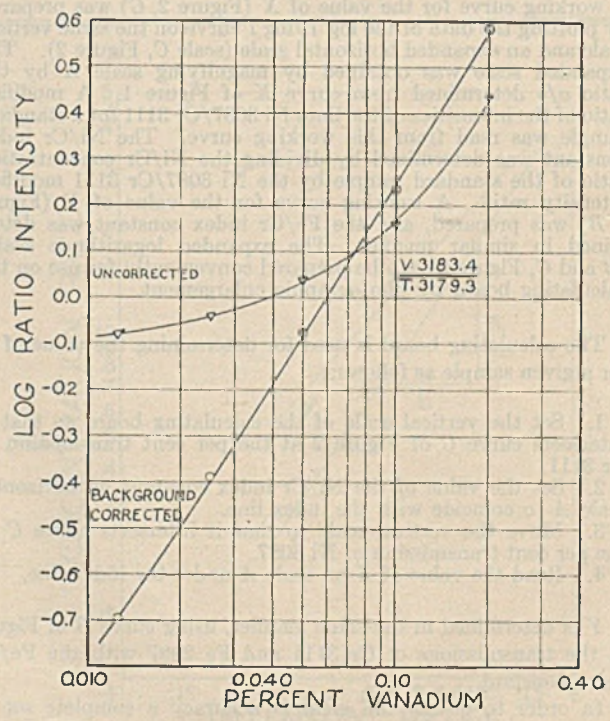


FIGURE 1. WORKING CURVE FOR VANADIUM IN TITANIUM DIOXIDE

Table I. Typical Results of Titanium Dioxide Analysis

Antimony Added	Antimony Found	Chromium Added	Chromium Found	Vanadium Added	Vanadium Found	Iron Added	Iron Found
%	%	%	%	%	%	%	%
1.00	1.00	0.100	0.112	0.200	0.192	0.100	0.090
	0.67		0.073		0.171		0.114
	1.10		0.101		0.120		0.109
	0.95		0.111		0.150		0.111
	0.92		0.096		0.170		0.106
Av.	0.93		0.099		0.161		0.106
0.50	0.57	0.050	0.056	0.100	0.106	0.050	0.056
	0.48		0.045		0.097		0.046
	0.49		0.051		0.089		0.054
	0.55		0.045		0.091		0.050
	0.61		0.059		0.092		0.055
Av.	0.54		0.051		0.095		0.052
0.25	0.22	0.025	0.024	0.050	0.050	0.025	0.049
	0.28		0.025		0.043		0.022
	0.26		0.026		0.057		0.033
	0.29		0.025		0.040		0.019
	0.35		0.030		0.042		0.037
Av.	0.28		0.026		0.046		0.032
0.12$_5$	0.15	0.013	0.014	0.025	0.025	0.0125	0.016
	0.15		0.012		0.024		0.013
	0.13		0.013		0.034		0.009
	0.05		0.008		0.022		0.012
	0.14		0.008		0.029		0.009
Av.	0.12		0.011		0.027		0.012
Av. error (5 detns.)	7.8		4.8		10.1		10.5

coated by dipping them in the molten solution. In addition to providing a simple means of preparing homogeneous standards, the procedure eliminates the undesirable sputtering characteristics of the titanium dioxide arc.

Preparation of Standard Samples

Exactly 100.0 grams of spectroscopically pure potassium bisulfate and 5.00 grams of pure titanium dioxide were fused together in a 115-mm. porcelain dish over a Méker burner. To the melt were added 0.0600 gram of antimony trioxide, 0.0352 gram of ferrous ammonium sulfate, 0.0240 gram of vanadous chloride, and 0.0141 gram of potassium dichromate. The resulting clear melt contained 1.00 per cent antimony, 0.20 per cent vanadium, 0.10 per cent iron, and 0.10 per cent chromium (all based on titanium dioxide). After the melt had been thoroughly stirred, smooth-faced graphite electrodes were dipped therein. The ends of the electrodes were barely touched to the surface of the melt. A smooth thin coat of salt was assured by preheating the electrodes before immersion of their tips, and by shaking off the excess salt immediately after their removal from the melt. A thin vitreous coating was found to lead to better reproducibility, since electrode separations could be more accurately controlled.

When the remaining melt had solidified, it was crushed and weighed. One half of the crushed salt was re-fused with 50.0 grams of potassium bisulfate and 2.50 grams of titanium dioxide. In similar fashion, succeeding standard melts and electrodes were prepared, each containing one half the impurity content of the former.

Spectra were recorded by a large quartz Bausch & Lomb Littrow spectrograph set for the region 2500 to 3400 Å. The electrodes, held in water-cooled nickel electrode holders, were accurately adjusted to a separation of 1.0 mm. and arced at 4.5 amperes (2200-volt alternating current arc). The exposure time was 1 minute (no pre-arc), with a spectrograph slit of 30 microns. Eastman Spectrum Analysis No. 1 plates, calibrated by means of an iron arc and a rotating stepped sector, were developed for 2 minutes at 18° C. in D-72.

Treatment of Data

The line pairs chosen for the construction of working curves were: Sb 2598/Ti 2590.3 Å., V 3183.4/Ti 3179.3 Å., Fe 3020.6/Ti 3002.7 Å., and Cr 3021.6/Ti 3002.7 Å. It was found desirable, in the interests of precision, to apply background corrections to all lines except Sb 2598 Å. and Ti 2590.3 Å., which lie in a region of low background. The background correction, discussed by Pierce and Nachtrieb (*3*), consists in converting line and background transmissions (or densities) into relative intensities, and subtracting the background intensities from the line plus background intensities for both analysis and internal standard lines. The logarithms of the background-corrected line intensity ratios (analysis line ÷ internal standard line) are then found to be a linear function of the logarithms of the concentrations down to rather low concentration limits. The fact that the working curves depart from linearity at low concentrations indicates that the titanium dioxide was not pure. The residual concentrations of impurities in the titanium dioxide could be determined by a method of successive approximations (*2*) or by construction of intensity-concentration graphs (*1*, *3*).

Figure 1 shows the effect of background correction on the vanadium working curve; the increased sensitivity (slope) occasioned by the correction is typical. Moreover, it is noteworthy

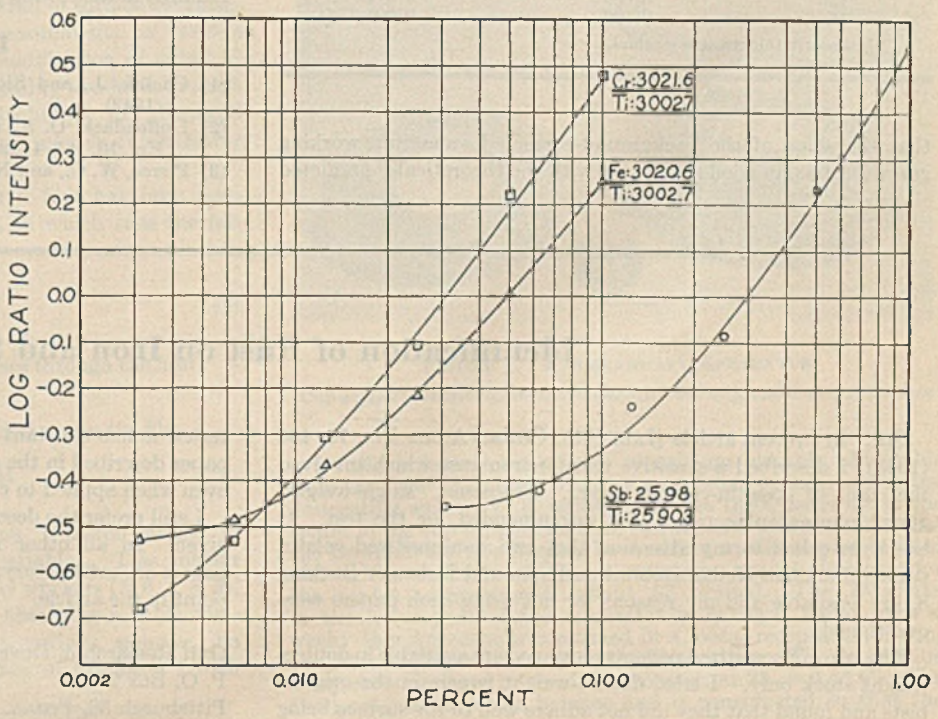


Figure 2. Working Curves for Iron, Chromium, and Antimony in Titanium Dioxide

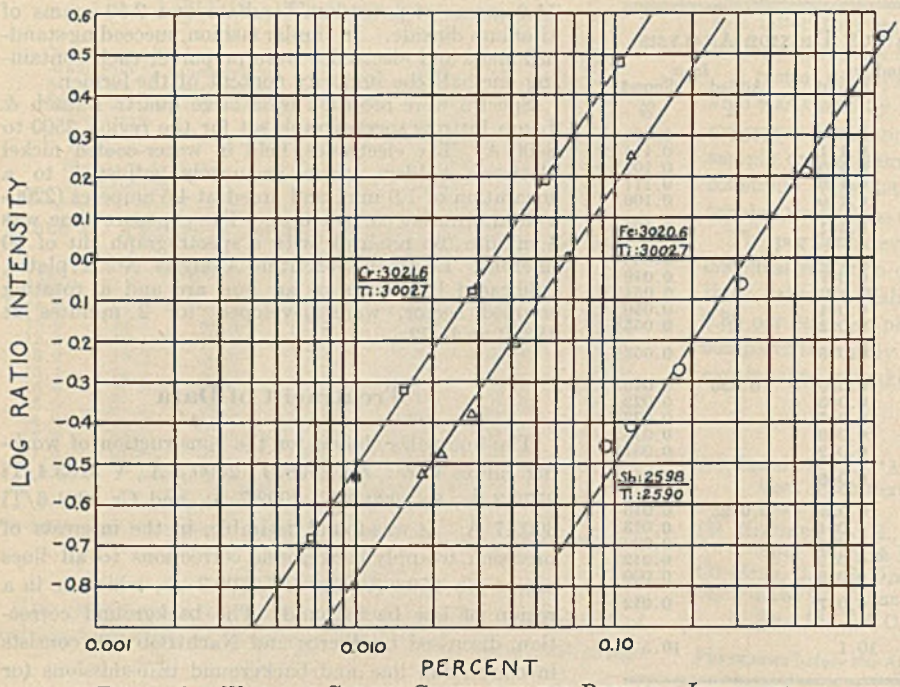


FIGURE 3. WORKING CURVES CORRECTED FOR RESIDUAL IMPURITIES

value of unity. Figure 2 shows the working curves obtained for antimony, iron, and chromium. Figure 3 shows the final working curves, obtained from the curves of Figure 2 by correction for the residual impurities in the titanium dioxide.

Table I lists the results of four analyses in quintuplicate for each of the elements, together with the average deviation from the true values. The time required for the eighty determinations was about 5 hours.

Table II gives a comparison of results for iron in sixteen samples of titanium dioxide by chemical analysis and by the spectrochemical procedure described in this paper.

TABLE II. COMPARISON OF SPECTROCHEMICAL AND CHEMICAL ANALYSES OF IRON IN TITANIUM DIOXIDE

Sample No.	Iron Found: Spectrochemical	Iron Found: Chemical[a]
	%	%
1	0.003	0.003
2	0.006	0.006
3	0.016	0.016
4	0.027	0.033
5	0.029	0.029
6	0.002	0.003
7	0.006	0.007
8	0.005	0.005
9	0.002	0.004
10	0.003	0.003
11	0.005	0.004
12	0.004	0.004
13	0.009	0.011
14	0.004	0.006
15	0.009	0.012
16	0.006	0.006

[a] Colorimetric thiocyanate method.

that the slope of the background-corrected vanadium working curve is 1.063, in good agreement with the theoretically predicted

Summary

A technique has been described for the analysis of powders which may be dissolved in molten potassium acid sulfate. The method has been applied to the determination of antimony, vanadium, chromium, and iron in titanium dioxide, analysis conditions and working curves for which are presented. The advantages derived from the application of background corrections are illustrated by working curves for a vanadium line.

The fused salt technique of preparing samples and standards may be extended to many other substances which are difficult to dissolve in the common solvents or to are as such. The selection of a flux in a particular case will be determined largely by solubility considerations and by its suitability as a spectroscopic buffer.

Acknowledgment

The authors wish to express their appreciation to Leavitt Gard and to John Darby for carrying out the chemical analyses required in the testing of the method.

Literature Cited

(1) Cholak, J., and Story, R. V., *J. Optical Soc. Am.*, **32**, 502–5 (1942).
(2) Duffendack, O. S., and Wolfe, R. A., IND. ENG. CHEM., ANAL. ED., **10**, 161–4 (1938).
(3) Pierce, W. C., and Nachtrieb, N. H., *Ibid.*, **13**, 774–81 (1941).

Identification of Rust on Iron and Steel

SIR: In a recent article [IND. ENG. CHEM., ANAL. ED., **15**, 464 (1943)] I described a sensitive test for iron rust which involved the use of gelatin-coated paper. Processed, single-weight, glossy photographic paper was recommended for the test. It has been called to my attention that two nonsensitized gelatin papers, Eastman Kodak Imbibition Paper and Defender Backing S, are available and my reasons for not using such papers were questioned.

The two nonsensitized papers mentioned are available in double-weight stock only. I tried double-weight papers in the original tests and found that they did not adhere well to the surface being tested unless constant pressure was applied. The single-weight paper described in the article adhered perfectly without pressure, even when applied to curved surfaces of short radius.

I still prefer the desensitized single-weight paper for the reason given. In all other respects the double-weight nonsensitized papers are satisfactory. I regret that I neglected to mention this point in the article.

RALPH O. CLARK

Gulf Research & Development Co.
P. O. Box 2038
Pittsburgh 30, Penna.

Determination of Permeability of Agricultural Spray Coatings to Water Vapor

C. L. COMAR[1] AND E. J. MILLER, Michigan Agricultural Experiment Station, East Lansing, Mich.

A simple empirical method capable of yielding reproducible permeability measurements on films formed from wax and oil-spray emulsions of the oil-in-water type is described. The experimental conditions are easily duplicated. For the type of film studied a linear relationship exists between moisture impedance and film thickness. Advantages in the use of paraffin wax are demonstrated and the role of bentonite in the emulsion is indicated.

IN THE course of studies on the development and use of emulsions for spraying plant materials to retard desiccation, it became necessary to determine the efficiency of films formed from such spray materials in reducing the passage of water vapor. Information on the interrelationships of film permeability, film thickness, and the chemical composition of the emulsion is indispensable for the formulation of spray materials having desirable physical characteristics. Methods described in the literature were not directly applicable to the type of material under consideration here from the point of view of preparation of the test film, precision of the measurements, and reproducibility of experimental conditions. It was therefore necessary to develop a more satisfactory procedure.

Examination of the literature (*3*), as well as laboratory experience, has indicated the many difficulties encountered in obtaining reproducible values from what appears to be a simple physical measurement. Uncertainties may be due to irregularities in the test film or to lack of consideration of the factors involved in the permeability measurement. In the method herein presented these difficulties have for the most part been eliminated by an exacting uniformity in procedure. The method is a simple one, and although designed for a specific kind of surface covering, it should be generally applicable to measurements on various types of coatings, perhaps with slight modification of detail in some cases. The apparatus required is readily available to most laboratories and if all precautions in technique are observed there should be no difficulty in obtaining reliable comparative measurements.

The passage of water vapor through a film has been considered as an ordinary diffusion process, in which case the following relation obtains:

$$M = kAt\frac{(P_1 - P_2)}{l} \qquad (1)$$

where M = mass of water vapor that passes through the film
A = area of film surface
t = time
$P_1 - P_2$ = water vapor pressure differential across the film
l = film thickness
k = constant for the film material

In actual practice, however, Equation 1 is too simple to express the relationship between the variables. This matter has been recognized and discussed by many workers (*1, 2, 4, 8, 9*). Since the interest here was primarily in obtaining reliable comparisons between the permeabilities of various samples, no attempt was made to establish the validity of Equation 1 with respect to all the variables; rather, emphasis has been placed upon a study of the precision of permeability measurements as a function of experimental technique with a view towards arbitrary standardization of conditions. It was desirable that the experimental conditions be easily maintained and readily capable of duplication, and that factors which might cause uncertainties in the results be controlled. Such considerations led to a satisfactory procedure.

[1] Present address, University of Florida, Gainesville, Fla.

Experimental

PERMEABILITY MEASUREMENT. A critical survey of procedures used for this type of measurement up to 1937 has been published by Carson (*3*) and the more recent experimental methods have been reviewed by Lishmund and Siddle (*9*). In the method reported here the material to be tested is fastened over the mouth of a cup containing water and the loss of moisture through the sample to a drier atmosphere is determined by weighing.

The Payne permeability cup (*10*), which is available commercially, was used for the measurements. It was found convenient, however, to replace the usual clamps with steel ones, since under constant usage the threads on the originals were easily stripped. Desiccators of the type shown in Figure 1, with an inside diameter of 250 mm., were used. The cups are supported within the desiccator by a 1.9-cm. (0.75-inch) plywood board which accommodates four cups and has a receptacle for desiccant in the center (see Figure 1). The cup support has an outside diameter of 233 mm.; the diameter of the center receptacle, which is made of metal, is 100 mm., and it is 7 to 8 mm. deep. The holes for the cups are 40 mm. in diameter and are symmetrically placed, the center of each hole being 25 mm. from the outside edge. The relation between cup position and the surface of the desiccant is then essentially the same for each determination.

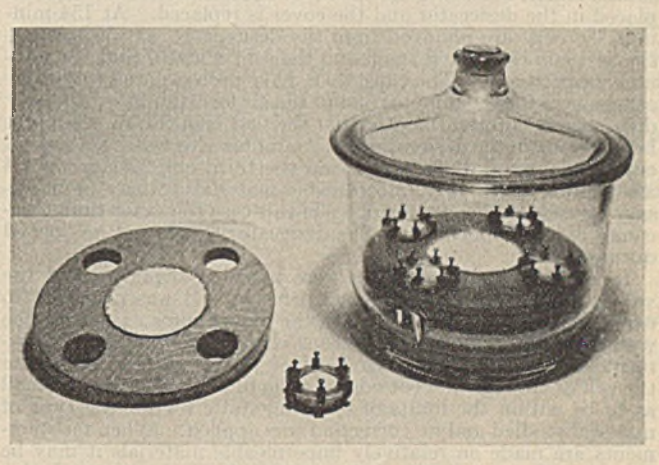


FIGURE 1. PERMEABILITY APPARATUS
Cup support containing desiccant, permeability cup, and loaded desiccator

Dowflake (a technical grade of calcium chloride obtainable from the Dow Chemical Company) was found satisfactory as the desiccating agent. It is necessary to use fresh desiccant in the center of the cup support for each run and it is recommended that the Dowflake for this purpose be stored in small completely filled and tightly closed bottles, each containing enough desiccant for the center compartment (about 57 grams). The Dowflake in the lower part of the desiccator is changed about once a week; this desiccant is contained in a metal receptacle for convenience and cannot be seen in the illustration. The desiccators themselves are kept in an air thermostat, at 25 ± 0.4° C. The thermostat is near the balance and is constructed so that the

desiccators need not be removed from it for the placing or removal of the cups.

Preliminary trials indicated that reproducible results could be obtained only if experimental conditions were constant. For this reason the following schedule was strictly maintained. Unless otherwise noted all measurements were made on the basis of a time period of 150 minutes; for the type of material studied no significant error was introduced by lack of attainment of the steady state of moisture interchange.

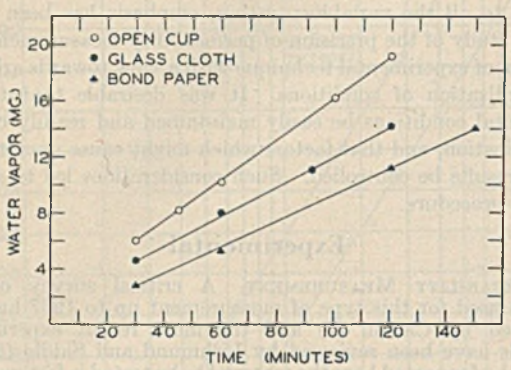


FIGURE 2. PERMEABILITY OF GLASS CLOTH AND BOND PAPER

Before the determination is started, distilled water, the small bottles of desiccant, and the cups are brought to 25° C. For each run, four samples of the film are used, three for the actual measurements and one for the counterpoise. A 5-ml. sample of distilled water is pipetted into each of three cups. Since all cups are of equal dimensions, the distance between the water surface and the film support is always the same. A film disk, with the coated side towards the dry atmosphere, is placed between the flanges of each of the four cups and the flanges are then clamped together tightly. The cup containing no water is used as the counterpoise. Fresh Dowflake is then placed in the center receptacle of the cup support inside the desiccator.

Starting at 0 minutes the first cup is weighed, at 1 minute the second and at 2 minutes the third; at 4 minutes the four cups are placed in the desiccator and the cover is replaced. At 154 minutes the cups are removed from the desiccator; at 156 minutes the first cup is weighed again and the others at 157 and 158 minutes, respectively. The cups, then, have been exposed to the dry atmosphere for 150 minutes and to the air for 6 minutes; the only difference in cup treatment is that the first is in the air 4 minutes before being in the desiccator and 2 minutes afterward, the second cup 3 before and 3 afterward, and the third cup 2 before and 4 afterward. With very impermeable materials it may be necessary to use a longer time period; in this case the exact timing becomes of less importance. The desiccator should not be opened during the run.

In all cases determinations were made in triplicate and the values usually agreed within ±3 per cent. It was necessary to use a counterpoise identical with the loaded cups except for the water, to eliminate any extraneous changes in weight. Experiment showed that the leakage between the sample and the rim of the cell or through the cut edges of the film support was so small as to be within the limits of experimental error for the type of material studied and no correction was applied. When measurements are made on relatively impermeable materials it may be necessary to use gaskets or other means of reducing rim and edge leakage.

PREPARATION OF FILMS. Satisfactory free films could not be prepared and a supporting material had to be employed. Glass cloth (*10*) and various types of paper were tried; the most consistent results were obtained with bond paper (Howard Bond, white, Sub. 24) and this was used for the work reported here. The water-marked parts of the sheet were not used.

The doctor blade technique could not be used because of the buckling of the film support caused by water absorption from the emulsion, and spraying or dipping yielded irregular films. Satisfactory films were prepared by the spinning method suggested by Gardner and Sward (*7*) and Gardner (*6*). The apparatus consisted essentially of a 7.5-cm. circular spinning table powered by a 0.125-horsepower motor. The speed could be varied by means of pulleys in steps from 430 to 3480 r. p. m. The film support was secured to the spinning table, covered with the solution from which the film was to be formed, and then spun for 30 seconds. Experiment indicated that the film thickness did not vary with the time of spinning between 10 and 500 seconds. The film was allowed to dry in air before measurements were made or before a second coat was applied.

The thickness of the film support was measured with a micrometer before spinning, and after the film had dried another measurement was made; the film thickness was then obtained by difference with an estimated accuracy of ±3 microns. The thickness value for each film was the average of three measurements halfway between the center and the outer edge of the test disk. All reported results represent the average of values obtained from 3 disks. In the case of viscous solutions the films were thicker at the center, but this was usually not significant. The film weight was determined by difference from the weight of the supporting disk and the weight of the disk plus the dried film; the values represent the weight of film covering the total area of the test disk (24 sq. cm.).

TABLE I. VARIATION OF MOISTURE IMPEDANCE, FILM THICKNESS, AND FILM WEIGHT WITH SPEED OF SPINNING

(Oil emulsion)

Speed	Moisture Impedance	Film Thickness	Film Weight
R. p. m.	*Hr. sq. cm./mg.*	*Microns*	*Mg.*
2270	0.43	49	119
1760	0.49	62	158
1390	0.55	82	207
1070	0.59	94	228
880	0.70	120	295

Experimental Results

The Payne permeability cup is designed to present an area of 10 sq. cm. for the passage of the water vapor. Although it has not been shown that the permeability is linearly proportional to the area, the results in this paper have been calculated on the basis of 1 sq. cm. for convenience in expression; since all data were obtained under the same conditions, no error in comparative results should be introduced by this procedure. Likewise the results have been calculated on the basis of water loss per hour, although unless otherwise noted the actual time period for the measurement was 150 minutes. The permeability is expressed therefore as milligrams of water vapor permeating through 1 sq. cm. area of film and supporting material in 1 hour under the specified experimental conditions. Where the results have been expressed as the reciprocal of the permeability, or the moisture impedance, the units are hour square centimeters per milligram. The reciprocal relation between moisture impedance and moisture permeability is analogous to that between electrical resistance and electrical conductivity. In any system, then, the impedance values should be additive while the permeabilities

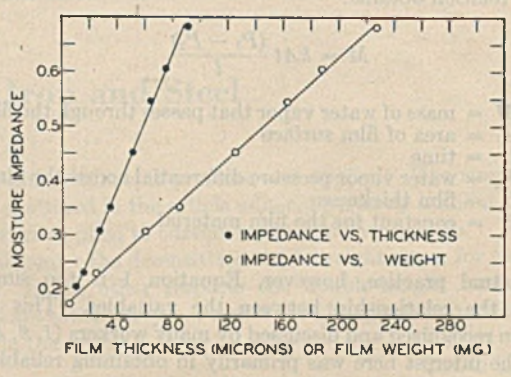


FIGURE 3. MOISTURE IMPEDANCE *vs.* FILM THICKNESS AND FILM WEIGHT

Oil emulsion

Table II. Oil Content and Moisture Impedance

(Oil emulsions)

Oil %	Moisture Impedance *Hr. sq. cm./mg.*
9	0.23
16	0.23
31	0.75
47	3.47

Table III. Effect of Wax on Moisture Impedance, Film Thickness, and Film Weight

(Oil emulsions)

Oil and Wax	Moisture Impedence *Hr. sq. cm./mg.*	Film Thickness *Microns*	Film Weight *Mg.*	Moisture Impedance per Unit Film Thickness
21.6% oil No wax	0.26	10	26	0.026
20.9% oil 0.7% wax	1.04	16	36	0.065
20.2% oil 1.4% wax	1.13	20	44	0.057
16.5% oil 5.1% wax	1.25	20	38	0.063

are not, just as in an electrical circuit the resistances are additive but the conductances are not. This has been discussed by Edwards (*5*).

Figure 2 presents the rate of loss of water vapor from the open cup as compared with that through glass cloth and bond paper. The ratios of the open cup values to those of the glass cloth agree closely with the numerical values of Payne (*10*). The permeability value obtained by extrapolation of the curves to zero time is probably due to the water loss during the 6-minute exposure to the air. The fact that the curve is linear up to a water vapor loss of almost 20 mg. from the open cup indicates that the efficiency of the desiccant has not been decreased by the absorption of that amount of moisture.

Table I presents the moisture impedance, thickness, and weight of a film formed from an oil-emulsion spray as a function of the speed of spinning. If these data are plotted it is apparent that the film thickness and weight are essentially linear with the moisture impedance.

Figure 3 presents the moisture impedance curves of films formed from an oil-emulsion spray. In this case the variation was obtained by changing the dilution of the emulsion rather than the speed of spinning. The linearity is in agreement with the results of other workers for some types of films under certain conditions (*11*, *13*). Wing (*12*) has reported the impedance of paint or enamel films to liquid water to be a linear function of film thickness. The moisture impedance obtained by extrapolation to zero thickness or weight agrees closely with the value for the film support alone. This type of information is important, since after linearity between moisture impedance and film thickness has been established for a given emulsion it is possible to interpolate the impedance at any desired thickness for comparative purposes.

Figure 4 presents the moisture impedance–per cent solids curves for the films formed from three different emulsions. Here also the impedance values approach that of the support material as the lower limit. The relative efficiency of these emulsions in retarding the passage of water vapor is obvious. At 12 per cent solids, wax emulsion A is about twice as efficient as emulsion B and 4 times as efficient as the oil emulsion; at 20 per cent solids, the efficiency ratios are 1.7 and 8.5, respectively.

Table II presents the moisture impedance of films formed from oil-emulsion sprays as a function of the oil content. It was necessary to increase the oil content to about 30 per cent to obtain a threefold increase in the impedance over the values obtained when less than 16 per cent oil was used. Increasing the oil content from 31 to 47 per cent gave over a fourfold increase in the impedance, but this range is not of practical interest because sprays containing such high percentages of oil tend to cause injury to the plants.

Table III shows the effect of incorporating small amounts of paraffin wax in an oil emulsion; it required only 0.7 per cent wax to increase the impedance fourfold, a more economical means of obtaining a greater impedance than the use of higher percentages of oil. There was an increase in the impedance per unit film thickness due to the presence of the wax.

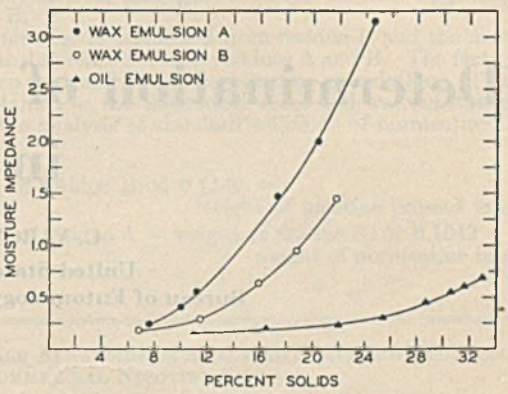


Figure 4. Moisture Impedance *vs.* Per Cent Solids

Table IV shows the effect of adding bentonite to an oil-emulsion spray. The bentonite increases the viscosity of the emulsion, and since the films are prepared under standard conditions, it is evident that an increased film thickness results. At the higher bentonite levels, however, the impedance per unit film thickness was markedly decreased. At the lower bentonite levels the decreased impedance per unit thickness was counterbalanced by the increased thickness, so that there was no net change in the impedance values in going from 0 to 2.1 per cent bentonite. Above 2.1 per cent bentonite the film thickness became the predominating factor and the over-all impedance values were increased.

The formulation of emulsions of given physical properties has been facilitated by information obtained from the above type of experiment.

Table IV. Effect of Bentonite on Moisture Impedance and Film Thickness

(Oil emulsions)

Bentonite %	Moisture Impedance *Hr. sq. cm./mg.*	Film Thickness *Microns*	Moisture Impedance per Unit Film Thickness
0.0	0.21	11	0.019
0.5	0.21	14	0.015
2.1	0.22	19	0.012
4.2	0.28	63	0.0045
5.2	0.47	125	0.0038

Acknowledgments

The authors wish to acknowledge the assistance of Robert Young, Almo Squitero, Howard Winters, and Anne Mandenberg with various parts of the experimental work. This research was supported by the Horace H. Rackham Research Endowment

of the Michigan State College of Agriculture and Applied Science for studies on the industrial utilization of agricultural products.

Literature Cited

(1) Abrams, A., and Brabender, G. J., *Paper Trade J.*, 102, No. 15, 32 (1936).
(2) Brabender, G. J., *Proc. 1st Food Conf. Inst. Food Tech.*, 1, 227 (1940).
(3) Carson, F. T., Natl. Bur. Standards, *Misc. Pub.* M127 (1937).
(4) Charch, W. H., and Scroggie, A. G., *Paper Trade J.*, 101, No. 14, 31 (1935).
(5) Edwards, J. D., Ind. Eng. Chem., Anal. Ed., 25, 846 (1933).
(6) Gardner, H. A., "Physical and Chemical Examination of Paints, Varnishes, Lacquers, and Colors", 9th ed., Washington Institute of Paint and Varnish Research, 1939.
(7) Gardner, H. A., and Sward, G. G., Ind. Eng. Chem., 19, 97 (1927).
(8) Kline, G. M., *J. Research Natl. Bur. Standards*, 18, 235 (1937).
(9) Lishmund, R. E., and Siddle, F. J., *J. Oil Colour Chem. Assoc.* 24, 122 (1941).
(10) Payne, H. F., Ind. Eng. Chem., Anal. Ed., 11, 453 (1939).
(11) Taylor, R. L., Hermann, D. B., and Kemp, A. R., Ind. Eng. Chem., 28, 1255 (1936).
(12) Wing, H. J., *Ibid.*, 28, 786 (1936).
(13) Wray, R. I., and Van Vorst, A. R., *Ibid.*, 25, 842 (1933).

Published with the permission of the Director of the Experiment Station as Journal Article 639 (n.s.).

Determination of Nicotine and Nornicotine in Tobaccos

C. V. BOWEN and W. F. BARTHEL
United States Department of Agriculture
Bureau of Entomology and Plant Quarantine, Beltsville, Md.

To determine nicotine and nornicotine in tobacco the total steam-volatile alkaloids are isolated by steam distillation from a strongly alkaline solution containing sodium chloride in excess. One aliquant of the distillate is treated with silicotungstic acid precipitating nicotine and nornicotine, and another is treated with nitrous acid, steam-distilled from an alkaline solution, and the nicotine precipitated as a silicotungstate. The nornicotine is determined by difference. Analysis of tobaccos differing widely in alkaloid content shows the rather common occurrence of nornicotine. Comparison with nicotine determinations by the conventional methods shows the unreliability of the heretofore accepted method of analysis when nornicotine is present.

A METHOD of analysis for nicotine and nornicotine when present together in solution has been presented by Markwood (*5*). It was developed for cases in which the alkaloids are already separated from interfering materials, and no procedure is given for obtaining the alkaloids from tobacco or tobacco products. To separate nicotine and nornicotine from the plant material, a steam distillation or continuous extraction with an organic solvent such as benzene is necessary. In view of the difficulty experienced in completely removing nornicotine from alkaline plant material by repeated extraction with benzene, ether, or gasoline, the method presented here resorts to a steam distillation, which completely separates both the nicotine and nornicotine from the sample.

This method is based on the assumption that anabasine or other steam-volatile secondary-amine alkaloids aside from nornicotine are absent; such alkaloids have been found only in traces in *Nicotiana tabacum*. The steam-volatile secondary-amine alkaloid has been identified as nornicotine by methylation in most of the samples analyzed.

The nornicotine is determined as the difference between the total steam-volatile alkaloid and the nicotine; consequently the analysis takes place in two parts. The first is an application to nornicotine of Burkat's method (*4*) for determining anabasine; it depends upon the complete steam distillation of nicotine and nornicotine from strong alkali solutions saturated with sodium chloride. The second part, which has been proved satisfactory by Markwood (*5*), involves treating the solution containing both alkaloids with nitrous acid and steam-distilling from an alkaline solution, whereby the secondary amines, including nornicotine, remain behind as nitrosoamines and the nicotine is obtained in the distillate.

Analysis of Standard Solutions

The standard solution of nicotine used for proving the method was prepared from a sample of nicotine that had been vacuum distilled, treated with nitrous acid to remove any secondary amine, and then steam-distilled from an alkaline aqueous solution.

The nornicotine used in preparing the standard nornicotine solution was identical with that used by Markwood (*5*), had been obtained from Robinson Medium Broadleaf, a strain of Maryland tobacco, and formed a picrate melting at 190–191° C. The absence of nicotine was established by treating a sample with nitrous acid and steam-distilling the aqueous solution after making it basic to phenolphthalein. No alkaloid was present in

Table I. Recovery of Nicotine and Nornicotine in Mixtures by Steam Distillation from Strong Alkali and Sodium Chloride

Composition of Mixture		Silicotungstate Residue[a]		
Nicotine	Nornicotine	Direct pptn.	Pptn. of distillate	Recovery
Mg.	*Mg.*	*Mg.*	*Mg.*	%
18.2	2.6	190.1	184.8[b]	97.2
14.5	4.5	172.7	170.3	98.6
10.9	6.2	152.2	155.0	101.8
7.7	7.2	138.9[b]	137.1	98.7
4.5	8.2	118.6[b]	117.9	99.4
			Av.	99.1

[a] Average of three determinations except where otherwise noted.
[b] Average of two determinations.

Table II. Analysis of Known Solutions of Nicotine-Nornicotine Mixtures

Nicotine			Nornicotine		
Calcd.	Found	Recovered	Calcd.	Found	Recovered
Mg.	*Mg.*	%	*Mg.*	*Mg.*	%
23.50	23.47	99.9	...	...	...
16.80	16.52	98.3	2.58	2.62	101.6
14.93	14.43	96.7	3.65	3.67	100.6
7.83	7.71	98.5	7.32	7.23	98.8
...	...	...	10.90	10.40	95.4
	Av.	98.4			99.1

the distillate. This nornicotine was steam-distilled and the distillate used in preparing the standard solution. Both solutions were standardized by the silicotungstic acid method.

The reliability of the silicotungstate precipitation for the determination of nornicotine is evidenced by the data presented in Table I.

The conventional method (*1*) for the steam distillation of nicotine specifies the use of a slight excess of alkali. When nornicotine is present, the time for complete distillation of the alkaloids is long and the volume of distillate consequently large, because the nornicotine distills only slowly from a weakly alkaline solution. When the distillation is made from a strongly alkaline solution, the speed of removal of alkaloids is greatly increased, and the addition of sodium chloride further hastens the process. Avens and Pearce (*2*) report that in their nicotine procedure excessively large amounts of sodium hydroxide give abnormally high results, but that they are due to something besides nicotine. This material is undoubtedly nornicotine, which did not distill readily at the lower alkali concentration. A comparison of the weights of the alkaloid-silicotungstate ignition residues of nicotine-nornicotine mixtures obtained by direct precipitation and by precipitating after steam distillation from strong alkali and sodium chloride (Table I) shows that an average recovery of 99.1 per cent may be expected. The alkali should be 30 per cent by weight and the sodium chloride in excess in order to have a saturated solution at all times during the distillation. When the fresh distillate and the residue from some of the samples were tested, the absence of alkaloid was indicated by no precipitation with silicotungstic acid.

When mixtures of the standard nicotine and nornicotine solutions were analyzed, one aliquot was steam-distilled directly to get all the steam-volatile alkaloid in the distillate, while another aliquot was treated with nitrous acid and then alkali before steam distillation. The alkaloids in the distillates were precipitated as silicotungstates and the precipitates ignited. The nornicotine was calculated from the difference in weights of the residues. The results shown in Table II indicate that such a procedure is satisfactory.

Analysis of Tobacco for Nicotine and Nornicotine

A 2.5-gram sample of powdered tobacco, a small piece of paraffin, 10 ml. of sodium hydroxide (30 per cent by weight), and 10 grams of sodium chloride are placed in the inner chamber of an improved steam-distillation apparatus (*3*) and steam-distilled into 3 ml. of dilute hydrochloric acid (1 + 4). Distillation is continued until a few drops of fresh distillate fail to give an opalescence when tested with silicotungstic acid solution. The distillate is concentrated by boiling (boiling stones added to prevent bumping) until the volume is less than 25 ml., and then transferred to a 25-ml. volumetric flask. Washings are added to the flask, and the liquid is brought to the mark. A 10-ml. aliquant is taken for precipitation of the nicotine and nornicotine with silicotungstic acid solution (12 per cent) according to the A. O. A. C. procedure for nicotine (*1*). The silicotungstate precipitate is filtered through a tared Gooch-type crucible and ignited, giving residue A. Another 10-ml. aliquant is made neutral to phenolphthalein, and 2 ml. of acetic acid (30 per cent) and 0.5 gram of solid sodium nitrite are added (*5*). After standing at room temperature for 20 minutes, this solution is made slightly alkaline with sodium hydroxide and steam-distilled into 3 ml. of hydrochloric acid from the improved distillation apparatus as used above. The distillate contains the nicotine, which is precipitated, filtered, and ignited as for the first aliquant, giving residue B.

The nicotine is calculated from residue B and the nornicotine from the difference between residues A and B. The factor 0.1042 has been assigned to the nornicotine calculation. This factor was obtained as the theoretical value and was substantiated by use in the analysis of standard solutions of nornicotine.

Weight of residue B × 0.1140 = weight of nicotine present in aliquant

(Weight of residue A − weight of residue B) × 0.1042 = weight of nornicotine in aliquant

Table III. Steam-Volatile Alkaloids in Analyzed Samples of Tobacco and Commercial Nicotine Sulfate

Sample	Calculated as Nicotine and Nornicotine, Proposed Method			Calculated as Nicotine	
	Nicotine	Nornicotine	Total	Proposed method	A. O. A. C. method[a]
	%	%	%	%	%
Nicotiana tabacum					
Robinson's Maryland Medium Broadleaf, Sample 1[b]	0.34	1.71	2.05	2.16	0.76
Cash, flue-cured[c]	0.70	2.40	3.10	...	...
Robinson's Maryland Medium Broadleaf, Sample 2[c]	0.98	2.16	3.14	3.32	1.77
Burley, Halley[c]	1.23	1.41	2.64	2.78	2.14
Turkish, Xanthi, American grown[c]	6.59	1.28	7.87	...	...
Maryland-Connecticut Broadleaf[c]	2.22	0.49	2.71	2.75	2.78
N. rustica					
Sample 1	4.57	0.99	5.56	...	...
Sample 2 (Brasilia)	6.71	0.66	7.37	...	...
Commercial nicotine sulfate					
Sample 1[c]	34.75	4.54	39.29	39.70	39.70
Sample 2	38.82	1.10	39.92	40.02	39.75

[a] Determined elsewhere.
[b] Source of nornicotine used to prepare standard solution.
[c] Steam-volatile secondary-amine alkaloid identified as nornicotine by methylation.

The results of analyses of several samples of tobacco (*Nicotiana tabacum*), *N. rustica*, and commercial nicotine sulfate by this method are given in Table III. For some of the samples the table gives the total steam-volatile alkaloid calculated as nicotine both by the proposed method and by the A. O. A. C. method. Upon calculation of the alkaloids as nicotine and nornicotine, an unexpectedly widespread occurrence of nornicotine is indicated. It is also evident that when nornicotine is present the accepted method of analysis cannot be depended upon to give reliable results.

Although the improved steam-distillation apparatus was used in the proposed procedure, the conventional steam-distillation apparatus may be substituted with a resulting increase in time required for distillation.

Literature Cited

(1) Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis, 5th ed., p. 64 (1940).
(2) Avens, A. W., and Pearce, G. W., Ind. Eng. Chem., Anal. Ed., 11, 505–8 (1939).
(3) Bowen, C. V., and Barthel, W. F., *Ibid.*, 15, 596 (1943).
(4) Burkat, S., *Farm. Zhur.*, No. 41, 228–34 (1937).
(5) Markwood, L. N., *J. Assoc. Official Agr. Chem.*, 26, 283–9 (1943).

Sensitive Indicator for Volumetric Determination of Boiler Feedwater Alkalinity

HARRY FLEISHER, Federal Works Agency, Public Buildings Administration, Washington, D. C.

IN A lime-soda process for the treatment of raw water for boiler consumption, accurate laboratory tests on the effluent water provide the fundamental bases for efficient softener control. Volumetric determinations of treated water alkalinities form a most important phase of such laboratory tests, and the chemist should have available accurate and positive color indicators in order to make the analyses with the highest degree of precision.

An ideal indicator would show a sharp point of color transition coincident with the equilibrium point for the completion of the neutralization reaction. This point was computed for boiler feedwaters at about 4.89 by interpolating the data reported by Cooper (*2*) to a millimolarity of 0.333, the average sodium carbonate content of such waters.

The use of methyl orange for alkalinity titrations of water has received almost universal application and has been adopted as standard (*1*). This indicator, however, possesses a color change rather difficult to detect and, more important, has been shown by Cooper (*2*) to have a pH color response in disagreement with the equivalence point for complete neutralization.

The reduction of such indicator uncertainty has been reported by various investigators through the application of mixtures of indicators and of indicators and dyes, and these mixtures are described in the literature (*5*). In the latter type of mixture, the dye is added to supplement the color of the indicator and produce a color other than that of the indicator itself. For example, an inert dye, xylene cyanole FF, has been proposed by Hickman and Linstead (*3*) to augment methyl orange, giving a green color with the alkaline yellow of the indicator and a magenta red color with the acid orange. The application of this mixture to feedwater titrations is subject to criticism in that the pH of the color transition is 3.8, according to Willard and Furman (*7*), making the indicator less suitable than methyl orange itself.

Johnson and Green (*4*) have investigated the use of an alcoholic solution of methyl red and alphazurine (*6*) for alkalinity determinations, and this mixture was applied as an indicator in titrating treated water alkalinities at the laboratory of the Federal Central Heating Plant, Washington, D. C. A modification in the ratio of dye to indicator was made to improve the color transitions. The mixture showed great promise, but after standing several weeks the methyl red separated from solution and the color response of the indicator became less pronounced. These difficulties were eliminated by substituting the sodium salt of methyl red (dimethylaminobenzene sodium carbonate) in place of the methyl red and water in place of the alcohol. The aqueous solution was found to be stable and its use avoided the necessity of using alcohol. The indicator was prepared by dissolving 0.45 gram of methyl red sodium salt and 0.55 gram of alphazurine in 1 liter of distilled water. The pH value of the solution was observed to be approximately 7.3.

The indicator was checked for accuracy by titrating 100 ml. of a solution of sodium carbonate (*M* 0.00033) with 0.033 *N* hydrochloric acid, using the usual "double end-point" method with 3 drops of phenolphthalein indicator for the first end point and 3 drops of the prepared indicator for the second end point. Potential changes were measured with a commercial glass electrode vacuum-tube pH meter (Table I). Color transition and maximum potential deflection were observed to be in good agreement with the computed equivalence point for the neutralization reaction, and the volume of acid required for the second titration end point was double that required for the first end point, all of which indicated accurate results.

The pH values of the indicator color changes were determined in a series of adjusted solutions of potassium acid phthalate (0.02 *M*) and showed slightly lower values—viz., 4.8 for the gray and 4.6 for the purple gray. However, in dilute unbuffered feedwaters, this small discrepancy should not materially affect the accuracy of the determinations.

The sensitivity of the indicator is evidenced by a color change from green gray to gray with the addition of one drop of acid (approximately 0.05 ml.), and gray to purple gray with a second drop. The color transitions are sharp and distinct and show sufficient contrast to make the changes easily distinguishable. The gray intermediate color gives ample warning of the approaching purple gray which is used as the end point of the titration.

The effect of neutral salts on the indicator was determined by titrating with 0.03 *N* hydrochloric acid several prepared feedwaters containing large quantities of such salts. The solutions used in the experiment were (1) 100 ml. of 0.00033 *M* sodium carbonate containing no salts, (2) 100 ml. of 0.00033 *M* sodium carbonate containing 400 p. p. m. of sodium chloride, (3) 100 ml. of 0.00033 sodium carbonate containing 400 p. p. m. of sodium sulfate, and (4) 100 ml. of 0.00033 *M* sodium carbonate containing 400 p. p. m. of sodium chloride and 400 p. p. m. of sodium sulfate. The titration of all four solutions to the indicator end point (purple gray) required 2.00 ml. (±0.025 ml.) of 0.033 *N* hydrochloric acid. The pH values for these end points ranged from 4.6+ for solution No. 1 to 4.9+ for solution No. 4. These results showed that although neutral salts increased the apparent pH (as indicated by the glass electrode) of the equivalence point by as much as 0.3 pH unit, the stoichiometric end point, as indicated by the color response, was not affected, since all four neutralizations required the same volumes of acid within experimental error. Hence the indicator appears suitable for titrating any feedwater containing salts within the limits shown

TABLE I. NEUTRALIZATION OF SYNTHETIC FEEDWATER

(100 ml. of 0.00033 *M* Na_2CO_3 titrated with 0.033 *N* HCl at 20° C.)

	0.033 *N* HCl Added *Ml.*	pH	ΔpH	Indicator	Observed Color
	0.0	9.87	...	Phenol-	Red
	0.1	9.78	0.09	phthalein	
	0.2	9.70	0.08		
	0.3	9.60	0.10		
	0.4	9.50	0.10		
	0.5	9.37	0.13		
	0.6	9.23	0.14		
	0.7	9.04	0.19		
	0.8	8.77	0.27		
	0.9	8.40	0.37		Pink
E. P.	1.0	7.50	0.90		Colorless
	1.1	7.15	0.35	Alphazurine-	Green
	1.2	6.85	0.30	methyl red	
	1.3	6.50	0.35	sodium salt	
	1.4	6.20	0.30		
	1.5	6.00	0.20		
	1.6	5.76	0.24		
	1.7	5.59	0.17		
	1.8	5.40	0.19		
	1.9	5.18	0.22		Green gray
E. P.[a]	2.0	4.68	0.50		Purple gray
	2.1	4.20	0.48		Purple
	2.2	3.75	0.45		
	2.3	3.56	0.19		

[a] 2 ml. of 0.033 *N* HCl is the stoichiometric as well as the actual volume (±0.05 ml.) required for neutralization.

ove, and most natural waters in this country are normally thin these limits.

Literature Cited

) American Public Health Association, "Standard Methods for Examination of Water and Sewerage", 8th ed., p. 65, New York, 1936.
) Cooper, S. S., Ind. Eng. Chem., Anal. Ed., 13, 466 (1941).
(3) Hickman, K. C. D., and Linstead, R. P., *J. Chem. Soc.* (London), 121, 2502 (1922).
(4) Johnson, A. H., and Green, J. R., Ind. Eng. Chem., Anal. Ed., 2, 2 (1930).
(5) Kolthoff, I. M., and Rosenblum, C., "Acid-Base Indicators", p. 173, New York, Macmillan Co., 1937.
(6) *Oil, Paint Drug Reptr.*, 126, No. 15, 21 (1934).
(7) Willard, H. H., and Furman, N. H., "Elementary Quantitative Analysis", p. 71, New York, D. Van Nostrand Co., 1933.

Determining the Mechanical Stability of Emulsions

A Rapid Quantitative Method

REYNOLD C. MERRILL, Jr.

Western Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Albany, Calif.

A rapid quantitative method for determining the mechanical stability of emulsions involves measuring the rate of separation of internal phase under a constant centrifugal force. The reciprocal of the initial rate of separation at a constant centrifuge speed has been taken as a quantitative index of the mechanical stability of the emulsion. The method has been applied to both water-in-oil and oil-in-water emulsions stabilized by lecithin, soaps, and vegetable gums. It gives results in a few hours apparently comparable to those obtained by more tedious methods involving other factors and requiring measurements over many months.

Definite effects of the age of the emulsion on its mechanical stability as determined by this method have been found in the case of soap and saponin-stabilized emulsions.

N SPITE of the great importance of emulsions in many industries at the present time and the recent rapid increase in e number of new emulsifiers commercially available, very few antitative data are given in the literature comparing the bility of various types of emulsions stabilized by different nts. This scarcity of numerical data is probably due to the k of rapid quantitative methods for measuring the stability of ulsions.

The concept of emulsion stability involves the resistance of the ulsion to destruction by various forces. Probably the best asure of stability is the rate of separation of internal phase . The forces involved in any practical case depend on the nditions to which the emulsion is to be subjected during use. This paper reports the development of a method for determining the resistance of emulsions to breaking under the mechanical stress of centrifugal force. Other destructive forces ich might be used are shaking, stirring, jarring, impact, heat-, and freezing and subsequent thawing. Probably the resances of a series of emulsions will vary in the same manner for ferent types of mechanical stresses. However, for practical rposes it is advisable always to determine the stability of the ulsion under conditions of use in order to ascertain whether a re rapid method than conditions of use is a valid measure.

Numerous methods have been used for measuring the effectiveness of emulsifiers. One consists of measurements of such ysical properties of the system as surface or interfacial tensions (*7, 19*), electrical charges (by electrophoresis), potential difference between disperse phase and dispersion medium, thickness of the protective film, viscosity, or specific gravity (*2*). All these quantities are undoubtedly of some importance in determining stability, but it is doubtful whether a single measurement of any of these properties constitutes, of itself, a determination of the stability of the emulsion. For example, the interfacial tension is probably an index of the ease of emulsification but is unreliable as a measure of the stability of the resulting emulsion (*20, 21*).

Another method of measuring effectiveness is the determination of either the maximum volume of disperse phase emulsified by a definite amount of emulsifier (*4*) or the smallest amount of emulsifier necessary to emulsify given volumes of different liquids (*8, 22*). These two methods measure the relative amounts of stabilizer and disperse phase necessary to produce an emulsion but do not measure its stability.

A third method consists of measuring the amount of salt necessary to separate the two phases (*16*). This method is not completely satisfactory as a measure of stability for different types of emulsions because the relative importance of electrical charge in determining stability may differ in various cases. Thus, emulsions in which stability is primarily due to the electrical charge on the droplets would probably appear less stable by this test than emulsions stabilized by a neutral film, although by other methods the two might appear equally stable.

A fourth method consists of the determination of the time required for a light to glow when the emulsion is placed between electrodes in series with a light bulb (*18*). This method also overemphasizes electrical factors contributing to stability and is applicable only to certain types of emulsions.

A fifth method is the determination of the time required for noticeable separation of phases on standing (*23*); and a sixth consists of measuring the variation with time of the droplet-size frequency (*6*) or interfacial area (*10*). A single size-frequency measurement is not sufficient because, as King (*9*) has pointed out, it appears that emulsion stability is not, in general, a function of the degree of dispersion. In utilizing this sixth method one should make sure that the change in droplet-size frequency actually represents phase separation and not merely an increase of droplet size to a larger, more stable form.

Other methods utilized for evaluating emulsions involve determining the oil content of horizontal layers of day-old emulsions (which involves creaming as well as breaking, *17*), observing the shapes of droplets of fungicidal emulsions dropped into water (*5*), measuring the toxicity to guinea pigs of dispersed bacterial toxin (which also involves the degree of dispersion of the emulsion and a possible detoxifying effect of the emulsifier, *15*), and the rate of saponification of oils (*13, 14*).

The methods that are fairly rapid are, in general, probably not valid measures of stability, whereas the more reliable methods, involving measurement of the separation of internal phase, are somewhat tedious and require measurements over a period of months and perhaps even years. Thus Berkman (*1*) mentions emulsions which show no signs of separation in five years. There is,

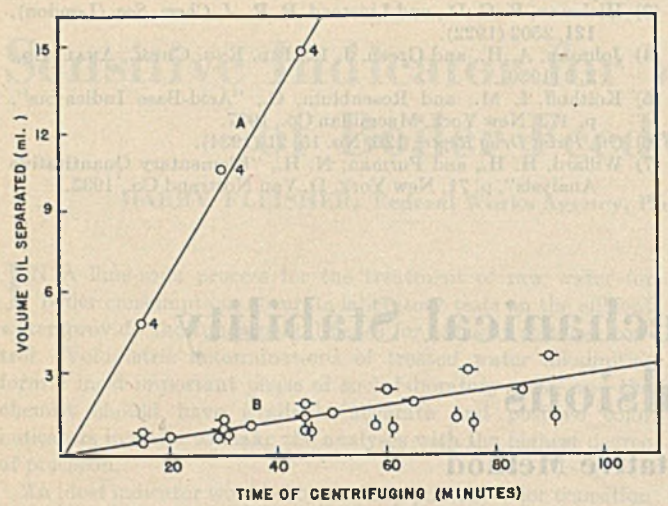


FIGURE 1. RATE OF SEPARATION OF BUTYL PHTHALATE FROM AQUEOUS EMULSIONS STABILIZED BY SODIUM ALEURITATE

Curve	Soap	Rate, Ml./Min.	K
A	0.1% sodium aleuritate (4 days old)	0.33	3.0
B	0.1% sodium aleuritate (fresh soln.)	0.030	33.0

The circles with different markings represent different runs on sodium aleuritate solutions varying in age by a few hours. Speed of centrifuging, 3000 r. p. m.

therefore, a definite need for reliable quantitative methods of measuring the stability of emulsions capable of giving results in a few hours. Since the rate of breaking of emulsions is the only reliable criterion of their stability, we have accelerated the breaking by centrifuging them in graduated tubes and have determined the rate of separation of internal phase at a constant speed of rotation. The reciprocal of the rate of phase separation is taken as a quantitative measure of the mechanical stability of the emulsion and is designated by K.

Experimental Method

The centrifuge used is a No. 1, Type SB International clinical centrifuge, equipped with horizontal heads carrying either four or eight 50-ml. tubes, a slide-wire resistance, and a Waltham tachometer reading up to 6000 r. p. m. The speed can be kept constant to about ±20 r. p. m. The distance from the center of rotation to the far end of the 9.5-cm. metal tubes used is 20.5 cm. The centrifugal force can be calculated from the formula

$$F = \frac{4\pi^2 n^2 mr}{3600 \times 980}$$

where F is the force, in multiples of the gravitational force, acting on a particle of mass m grams a distance of r centimeters from the axis of rotation at a speed of n revolutions per minute. The force acting on a particle in the center of the tube is 176, 1580, and 2280 times gravity at speeds of 1000, 3000, and 3600 r. p. m., respectively.

The emulsions were contained in graduated 40-ml. Pyrex, heavy-duty conical-bottom centrifuge tubes. During centrifuging the tubes were covered with a piece of sheet rubber held in place by a rubber band in order to minimize evaporation and aging effects on soap emulsions due to absorption of carbon dioxide from the air. The time of centrifuging was measured with an automatic electric timer equipped with a warning device. The volume of the dispersed phase which had broken from each emulsion was noted at successive intervals of 5 to 30 minutes and plotted as a function of time at a constant centrifuging speed. Care was taken in stopping the centrifuge to avoid convection currents which might otherwise have affected the results. Any temperature rise in the samples on prolonged centrifuging is so small as to be negligible.

Chemicals

The soaps of aleuritic (9,10,16-trihydroxypalmitic), palmitic, and stearic acids were made from the pure Eastman acids by neutralization in acetone solution with sodium ethylate for the sodium soaps, and with the corresponding hydroxide for ammonium and potassium palmitates. Sodium oleate was made from a technical oleic acid. Heavy metal soaps were made by precipitating them from hot solutions of the corresponding sodium soaps with a slight excess of the alkaline earth chloride. The precipitates were washed with hot water and dried in a vacuum desiccator.

The sodium palm-oil soap was made from an Ecuadorian palm oil (supplied by E. B. Kester, Western Regional Research Laboratory) in which the free fatty acids had an equivalent weight of 242. The saponin, cyclohexanol, lecithin, butyl phthalate, and castor oil were Eastman products. Aerosol OT, a trade name for the sodium salt of the dioctyl (2-ethylhexyl) ester of sulfosuccinic acid, is a pure wetting agent obtained from the American Cyanamid and Chemical Corporation. The benzene was the thiophene-free grade obtained from Eimer & Amend. The carbon tetrachloride, kerosene, tragacanth (U. S. P. No. 2), karaya (XXX grade), and acacia gums were commercial products. The pectin was a commercial citrus pectin with a jelly grade of 200.

Soap solutions were prepared immediately before using from freshly boiled distilled water. Accurately weighed amounts of solid soap were dissolved in sufficient water to give the desired volume.

Preparation of Emulsions

Emulsions were prepared by running the two liquids four or five times through a hand-operated aluminum emulsifier or homogenizer (Central Scientific, Catalog No. 70,180), in which a piston forces the liquids through six small slits between parallel plates of the 8-mm. nozzle, producing a rather effective shearing action. Except where otherwise stated, equal volumes of the organic liquid and of aqueous solutions of the stabilizing agent were emulsified. The concentrations of stabilizing agent given are based on the volume of aqueous solution, not on the total volume of emulsion. All experiments were carried out at room temperature.

For preliminary work on the method, emulsions were used in which the internal phase had a higher density than the dispersion medium, since volume readings at the bottom of the centrifuge tubes are easier to make and can be made more precisely. It was also thought that if the internal phase were heavier, the centrifugal force would accelerate breaking rather than creaming, thus making the results more significant. However, later work showed the method to be applicable even when the disperse phase is lighter than the dispersion medium. Dibutyl phthalate (density 1.0465) was chosen as the internal phase, since dye tests using methylene blue and Orange OT showed that it formed oil-in-water emulsions with the water-soluble soaps used as emulsifiers.

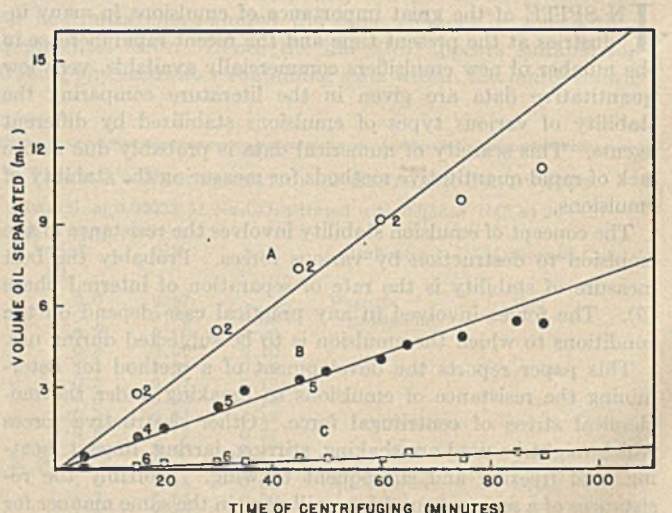


FIGURE 2. RATE OF SEPARATION OF BUTYL PHTHALATE FROM AQUEOUS EMULSIONS STABILIZED BY SAPONIN AND SODIUM PALM-OIL SOAP

Curve	Soap	Rate, Ml./Min.	K
A	0.1% saponin (4 days old)	0.150	6.0
B	0.1% saponin (fresh soln.)	0.070	14.6
C	0.1% sodium palm-oil soap	0.006	166.0

Speed of centrifuging = 3000 r. p. m.

Experimental Data and Discussion

Representative data obtained are shown in Figures 1 to 5, in ich volume of oil or internal phase separated is plotted as a ıction of the time of centrifuging. The rate of separation is ınd to be practically constant until about 60 to 75 per cent of internal phase has separated; above this region the rate ·reases markedly, although the whole curve does not appear be logarithmic. After visual inspection of the data, straight es corresponding to the initial rate were drawn so as to give the st fit of experimental points. Freshly prepared emulsions ıtaining unemulsified oil show separation at the same rate as completely emulsified systems.

The formula for the rate of separation of phases proposed by derer (*11*) does not apply either to the data of this investigation to all the previous data of Cheesman and King (*3*) obtained by asuring the rate of separation on standing.

Since the rate-of-separation curves appear to be similar in type the emulsions studied, it is sufficient for practical purposes take the initial rate of separation of disperse phase as an index the mechanical stability of the emulsion. Logically, it is prefer- e to call this a measure of the mechanical instability and to ıe the reciprocal of this rate as a measure of the mechanical bility. For rapid comparative studies only the relative ounts of internal phase separated after a constant arbitrary ıe of centrifuging need be measured.

Aging Effects

Emulsion stabilized by saponins and soaps show pronounced ng effects after being allowed to stand in a beaker covered with vatch glass, even though thoroughly re-emulsified by the pre- usly used technique immediately before centrifuging. The ent of aging varies with the stabilizing agent. The rate of aration of dibutyl phthalate at a speed of 3000 r. p. m. from ulsions stabilized by 0.1 per cent sodium aleuritate increases fold (from 0.030 to 0.33 ml. per minute) in 4 days, whereas m those stabilized with 0.1 per cent saponin the rate little re than doubled (from 0.070 to 0.150 ml. per minute) in the ıe time. This rapid aging of sodium aleuritate solutions ac- nts for the slightly different rates obtained in different runs emulsions from supposedly fresh solutions whose age actually ied by as much as 6 or 8 hours. Different points from the ıe run form approximately straight lines when plotted as in ure 1. The aging effect is probably due at least partly to orption of carbon dioxide and subsequent hydrolysis of the p. The acid soaps formed are evidently much less effective ilizing agents. Saponin solutions also decompose on stand- . Such pronounced aging effects were not obtained with ılsifiers which are stable when exposed to air, nor would it be ected that they would be obtained with soap emulsions kept losed vessels free from carbon dioxide.

Reproducibility of Method

As an indication of the reproducibility of the centrifugal thod in its present state, the values from five independent s on butyl phthalate—0.1 per cent sodium aleuritate emulsions ed for 4 days and then re-emulsified)—are presented. The ounts of butyl phthalate separated at the end of various ods at 3000 r. p. m. were as follows:

r 15 Min.	After 30 Min.	After 45 Min.	After 60 Min.
Ml.	*Ml.*	*Ml.*	*Ml.*
4.0	11.0	16.0	17.1
5.2	10.4	14.9	17.1
5.1	10.2	14.4	17.1
4.9	11.1	14.5	
4.6	10.4		
4.8	Av. 10.6	Av. 14.9	Av. 17.1

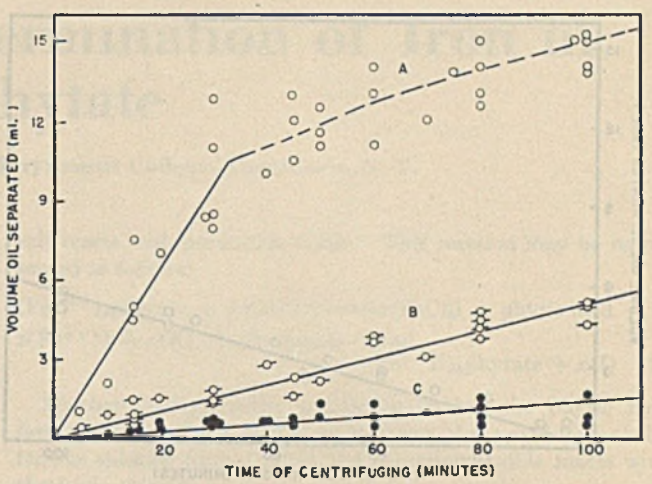


FIGURE 3. RATE OF SEPARATION OF BUTYL PHTHALATE FROM AQUEOUS EMULSIONS STABILIZED BY PECTIN AND VEGETABLE GUMS

Curve	Stabilizer	Rate, Ml./Min.	*K*
A	1% karaya gum	0.31	3.2
B	0.3% tragacanth	0.051	20.0
C	1% pectin	0.014	71.0

Speed of centrifuging = 3600 r. p. m.

The average values are used in Figure 1. Data for the other solutions and figures were obtained from three to six independent runs.

Comparison of Emulsifiers

Sodium palm-oil soap forms more stable emulsions with dibutyl phthalate than either saponin or sodium aleuritate does when compared at 0.1 per cent. Under the same centrifugal force, palm-oil-soap–stabilized emulsions show phase separation at about one fifth of the rate of fresh sodium aleuritate emulsions and about one tenth of the rate of fresh saponin emulsions. Butyl phthalate emulsions stabilized by 0.1 per cent vegetable gums are of larger droplet size and break much more rapidly than those stabilized by soaps. Lecithin emulsions are very stable and behave more like soap-stabilized emulsions than those stabilized with pectin or vegetable gums.

Figure 3 again shows the apparent constancy of the initial rate of separation of butyl phthalate from pectin, karaya, and tragacanth emulsions and the rapid decrease in rate after about 60 per cent of the karaya emulsion has broken. Karaya-stabilized emulsions break unevenly and as a result the error of reading is increased. This accounts for the somewhat greater spread of experimental points for karaya. Emulsions stabilized by gum acacia break even more rapidly than karaya emulsions. The order of decreasing stability of butyl phthalate emulsions is: tragacanth > pectin > karaya. Butyl phthalate emulsions stabilized by gum acacia are less stable than those stabilized by karaya.

Emulsions stabilized by 0.3 per cent lecithin appear to lose butyl phthalate at the constant initial rate of 0.057 ml. per minute (Figure 4). Lecithin is about equivalent to tragacanth as an emulsifier for dibutyl phthalate, although perhaps not quite so good.

Emulsions with Less Dense Internal Phase

In order to see whether the centrifugal method can be used when the droplets are lighter than the dispersion medium, equal volumes of 0.1 per cent Aerosol OT solutions and the following organic liquids were emulsified and centrifuged at 3000 r. p. m.: benzene (density 0.879), cyclohexanol (density 0.945), castor

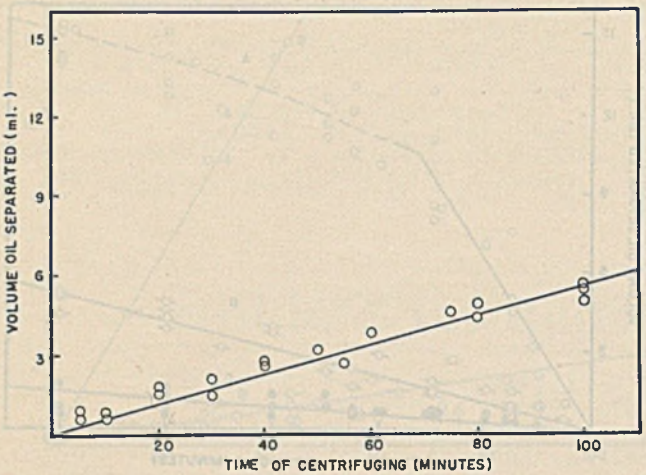


FIGURE 4. RATE OF SEPARATION OF BUTYL PHTHALATE FROM AN AQUEOUS EMULSION STABILIZED BY 0.3 PER CENT LECITHIN

Rate = 0.057 ml./minute. K = 18.
Speed of centrifuging = 3600 r. p. m.

oil (density 0.96), and mineral oil (density 0.85). Dye tests using Orange OT and methylene blue showed the emulsions to be of the oil-in-water type. On centrifuging, the liquid in the droplets separated at the top of the emulsion at the rate of 0.042, 0.069, and 0.31 ml. per minute for the cyclohexanol, benzene, and castor oil, respectively (Figure 5).

Castor oil, the density of which is nearest to that of water and therefore might be expected to separate more slowly than any of the others, separates the most rapidly. The fact that the separation rate of these emulsions is not determined only by the difference in densities of the phases is evidence that the centrifugal method measures emulsion stability. At any rate, the data show that the centrifugal method can successfully be used, even when the density of the disperse phase is less than that of the dispersion medium. However, care must be taken in interpreting comparative measurements obtained by this method on disperse phases of varying densities. Recently King (*9*) has presented evidence indicating that the stability on aging and resistance to salting out by potassium sulfate of aqueous emulsions with nonpolar liquids increased with decreasing difference between the densities of the two phases. The centrifugal method here described would probably yield the same result.

In addition to the liquids mentioned above the method has been used on aqueous soap-stabilized emulsions of cresol, aniline, toluene, kerosene, carbon tetrachloride, and mixtures of the last two. Experiments on water-in-oil emulsions of benzene and kerosene stabilized by heavy-metal soaps indicate that the method is applicable to these systems.

Comparison with Previous Investigations

After centrifuging at 3600 r. p. m. for 100 minutes the volumes of kerosene separated from a 50–50 emulsion stabilized by 0.005 N potassium, sodium, and ammonium palmitates were 1.3, 1.5, and 1.7 ml., respectively. This is the same order found by King and Mukherjee (*10*) for these same soaps at 0.001 N by the method of rate of decrease of interfacial area. Tests on mineral-oil emulsions stabilized by 1.66 per cent carbohydrate colloids indicated the order of increasing mechanical stability to be tragacanth << acacia < karaya ~ pectin. This is in qualitative agreement with the data of Lotzkar and Maclay (*12*) on these identical emulsions obtained by measuring the rate of decrease of interfacial area.

While the agreement between the rate of decrease of interfacial area and the centrifugal methods of measuring emulsion stability appears satisfactory, final proof of the value of either method for many practical purposes must await the comparison of data obtained by these methods with data on identical systems obtained by measuring the rate of separation on standing.

Preliminary tests have shown that increasing the centrifuge speed increases the rate of phase separation. It is possible that more experiments might show the rate of separation of the internal phases to be directly proportional to the applied centrifugal force. In that case, the reciprocal of the rate of separation divided by the average centrifugal force would be a preferable unit.

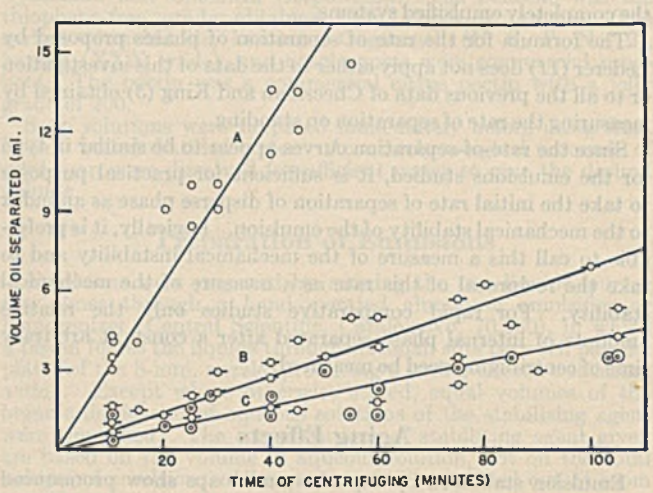


FIGURE 5. RATE OF INTERNAL PHASE SEPARATION FROM 0.1 PER CENT AEROSOL OT EMULSIONS

Curve	Organic Liquid	Rate, Ml./Min.	K
A	Castor oil	0.31	3.2
B	Benzene	0.069	14.5
C	Cyclohexanol	0.042	24.0

The double circles are points for cyclohexanol.
Speed of centrifuging = 3000 r. p. m.

Literature Cited

(1) Berkman, S., *J. Phys. Chem.*, 39, 527 (1935).
(2) Berkman, S., and Egloff, G., "Emulsions and Foams", p. 107, New York, Reinhold Publishing Corp., 1941.
(3) Cheesman, D. F., and King, A., *Trans. Faraday Soc.*, 34, 594 (1938); *Kolloid-Z.*, 83, 33 (1938).
(4) Donnan, F. G., and Potts, H. E., *Ibid.*, 7, 208 (1910).
(5) Goettsch, G., *Chem. Weekblad*, 26, 336 (1929).
(6) Harkins, W. D., and Beeman, N., *J. Am. Chem. Soc.*, 51, 1674 (1929).
(7) Hirose, M., *J. Soc. Chem. Ind. Japan*, 30, 823 (1927).
(8) Jajnik, N. A., and Ilahi, B., *Kolloid-Z.*, 37, 139 (1925).
(9) King, A., *Trans. Faraday Soc.*, 37, 168 (1941).
(10) King, A., and Mukherjee, L. N., *J. Soc. Chem. Ind.*, 58, 245T (1939).
(11) Lederer, E. L., *Kolloid-Z.*, 71, 61 (1935).
(12) Lotzkar, H., and Maclay, W. D., IND. ENG. CHEM., 35, 1294 (1943).
(13) McBain, J. W., Humphreys, C. W., and Kawakami, Y., *J. Chem. Soc.*, 1929, 2185.
(14) McBain, J. W., and Kawakami, Y., *J. Phys. Chem.*, 34, 580 (1930).
(15) Myers, G. N., *J. Hyg.*, 39, 529 (1939).
(16) Parsons, L. W., and Wilson, O. G., Jr., J. IND. ENG. CHEM., 13, 1116, 1119 (1921).
(17) Pchelin, A., and Novik-Bam, E., *Tsentral Nauch. Issledovatel Inst. Kozhevennoi. Prom. Sbornik Rabot*, No. 4, 111 (1934).
(18) Pick, L., *Chem. Obzor.*, 3, 202 (1928).
(19) Sazonoff, P. P., and Milnikoff, B. N., *Melliand Textilber.*, 8, 361 (1927).
(20) Simm, D. M., *J. Soc. Dyers Colourists*, 42, 212 (1926).
(21) Smith, R. C., and Dow, I. C., *J. Phys. Chem.*, 31, 1263 (1927).
(22) Tyutyunnikov, B., Kasyanova, N., and Plesehova, Z., *Allgem. Oel- u. Fette-Ztg.*, 28, 165, 185 (1931).
(23) White, M. G., and Marsden, J. W., *J. Phys. Chem.*, 24, 619 (1920).

Rapid Iodometric Determination of Iron in Iron Phytate

J. R. FOY AND J. B. THOMPSON[1], Marymount College, Tarrytown, N. Y.

A direct titration method makes it possible to determine iron in ferric phytate in about 20 minutes, whereas other methods adaptable to the product require several hours. The results obtained are highly reproducible. The method is more accurate than methods which involve considerable manipulation and possible loss.

FERRIC phytate has been proposed as a source of iron for the nutritional fortification of grain and cereal products. It is buff-colored compound, insoluble in water and dilute acids, ich does not accelerate the development of rancidity in foodffs. The iron is available to the animal organism and, since ytic acid is a natural constituent of cereals, no foreign matter ntroduced (*1*).

Since phytic acid forms iron salts of varying iron content, its alysis before incorporation into food products seems highly irable. The usual analytical procedures that might be aped to this compound are lengthy and offer considerable diffity. Ferric phytate, however, exhibits certain properties that ow a much more rapid determination of iron. Posternak (*5*) s shown that iron can be quantitatively split from the ferric ytate by strong alkali, producing the insoluble ferric hydroxide d the soluble alkaline phytate. By heating a sample of ferric ytate in a strong sodium hydroxide solution, filtering, washing e soluble sodium phytate from the ferric hydroxide, then disving the latter in hydrochloric acid, the iron content can be ermined by the usual Zimmerman-Reinhardt method. This ocedure, though satisfactory, is also tedious. The authors nd that ferric phytate is very soluble in concentrated hydroloric acid, and that under these conditions the ferric ion is dily available. It is sufficient, therefore, to add a potassium ide solution of the proper concentration to the strongly acid ution of the ferric phytate and, after dilution, to titrate the erated iodine with thiosulfate. Two samples of ferric phytate ve been analyzed for iron in this manner with very gratifying ults. Comparisons with determinations made by the Zimrman-Reinhardt method are satisfactory.

The iodometric determination of iron is usually considered unisfactory, as the reversibility of the reaction results in the alst immediate return of the blue starch-iodine color. Accord- to Kolthoff and Furman (*2*) the equilibrium expression for e iodometric titration of the ferric ion:

$$2Fe^{+++} + 2I^- \rightleftharpoons 2Fe^{++} + I_2$$

given as

$$\frac{[Fe^{++}][I_2]^{1/2}}{[Fe^{+++}][I^-]} = 21.1$$

In order to obtain quantitative results, it is recommended that e ferric ion be present as the salt of a strong mineral acid; that e solution be strongly acid, pH 1; and that an excess of the ide be present. However, when ferric phytate is titrated ometrically, in no case does the color return in less than 60 nutes, and 2 to 3 hours usually elapse before there is a recurnce. Thus the reduction of the ferric ion is of a more permant nature. Undoubtedly a soluble, but little dissociated, ferus potassium salt of phytic acid is formed which is not easily dized to the ferric state, as is the case when an inorganic ferric salt reacts with potassium iodide. This reaction may be represented as follows:

$$(Fe^{+++})_x\text{phytate} + 3x(HCl) \rightleftharpoons x(FeCl_3) + \text{phytic acid} \quad (1)$$

$$x(Fe^{+++}) + x(KI) + \text{phytic acid} \rightleftharpoons (Fe^{++}K)_x\text{phytate} + x(I) \quad (2)$$

Another (less probable) possibility is that the phytic acid formed in Equation 1 acts as an inhibitor of the oxidation of the ferrous chloride formed when the potassium iodide reacts with the ferric chloride.

The addition of phytic acid when iron is determined iodometrically in ferric ammonium sulfate delays the return of the blue starch-iodine color, just as in the case of the ferric phytate titrations. Although not yet completely worked out, it appears that the use of phytic acid could be adapted to the iodometric titration of other iron compounds.

Experimental

PREPARATION OF IRON PHYTATE SAMPLES. A solution of phytic acid was prepared from wheat bran by the method of Morrow and Sandstrom (*4*). Samples of iron phytate were prepared from this solution by precipitating with ferric chloride at pH 4 to 5, washing with dilute acid and finally with water, and drying in vacuum over phosphorus pentoxide.

Iron was determined in 1-gram samples by the accepted Zimmerman-Reinhardt method (*3*) after digestion with sulfuric and perchloric acids (Table I).

DIRECT TITRATION METHOD. Samples of 0.5 to 1.5 grams were transferred to 250-ml. iodine flasks, 10 ml. of hydrochloric acid were added, and the flasks were gently rotated to effect complete solution. Then 25 ml. of a 16 per cent potassium iodide solution were added, and the flask was quickly stoppered and twirled several times to ensure thorough mixing. The mixture was allowed to stand 5 minutes, 100 ml. of water were added, and the liberated iodine was titrated with 0.1 *N* sodium thiosulfate using starch indicator. The titrations were corrected for blanks run under identical conditions (Table I).

CALCULATION. 1 ml. of 0.1 *N* sodium thiosulfate is equivalent to 0.05585 gram of iron.

TABLE I. DETERMINATION OF IRON

No. of Determinations	Sample Weight	Iron Found: Direct titration	Iron Found: Zimmerman-Reinhardt
	Grams	%	%
		Sample A	
3	0.50	Max. 16.88 Min. 16.82 Av. 16.86	...
15	0.70	Max. 16.88 Min. 16.82 Av. 16.83	...
19	1.00	Max. 16.88 Min. 16.77 Av. 16.83	...
2	1.50	Max. 16.79 Min. 16.79 Av. 16.79	...
6	1.00	...	Max. 16.82 Min. 16.63 Av. 16.70
Av. of all determinations		16.82	16.70
		Sample B	
13	1.00	Max. 11.57 Min. 11.45 Av. 11.49	...
3	1.00	...	Max. 11.32 Min. 11.21 Av. 11.26

[1] Present address, Chicago Quartermaster Depot, Q.M.C. Subsistence Research Laboratory, Chicago, Ill.

TABLE II. BLANKS OBTAINED UNDER VARYING CONDITIONS

No. of Determinations	HCl	KI per 25 Ml. of Solution	Time of Standing	0.1 N $Na_2S_2O_3$, Average
	Ml.	*Grams*	*Min.*	*Ml.*
3	10	5.0	15	0.25
3	10	2.5	15	0.17
6	10	4.0	5	0.14
2	20	4.0	5	0.23
3	10	7.5	15	0.29

Discussion

Several factors must be carefully controlled. It is advisable to use samples of 0.7 to 1.00 gram, as then the volume of the thiosulfate used is well within the desirable titration range. The ratio of the sample weight in grams to the volume of hydrochloric acid in milliliters must be kept between 1 to 7 and 1 to 15 to ensure complete solution and yet avoid too great an excess of acid. The ratio of the volumes of the hydrochloric acid and potassium iodide solution must be kept between 1 to 2 and 1 to 3 to prevent the precipitation of the ferric phytate and yet maintain as low an acid concentration as possible. There is a blank liberation of iodine which depends upon the amount of potassium iodide and hydrochloric acid and the time of standing (Table II). Varying these three factors within the limits set up herein does not affect the determination, provided a correction is made for a blank run under identical conditions.

It is suggested that as a routine procedure the volume of the hydrochloric acid and potassium iodide solution be accurately measured by pipet and the time of standing be recorded on a stopwatch. It will then be necessary to run only one set of blanks for each batch of hydrochloric acid and potassium iodide for different times of standing. A 1 to 50 dilution of the thiosulfate in the blank titration will give more reliable values. If the blank titration is plotted against the time of standing, the correction to be applied to any determination can be read off the graph.

The results by the Zimmerman-Reinhardt method are slightly lower (0.1 to 0.2 per cent) than those by the direct titration method, which can undoubtedly be attributed to incomplete digestion and solution.

Literature Cited

(1) Andrews, J. S., Evans, L. H., and Huber, L. J., U. S. Patent 2,239,543 (Apr. 22, 1941).
(2) Kolthoff and Furman, "Volumetric Analyses", Volume II, p. 422, New York, John Wiley & Sons, 1929.
(3) Kolthoff and Sandell, "Textbook of Quantitative Inorganic Analysis", p. 570, New York, Macmillan Co., 1936.
(4) Morrow and Sandstrom, "Biochemical Laboratory Methods", p. 226, New York, John Wiley & Sons, 1935.
(5) Posternak, S., *Compt. rend.*, 168, 1216 (1919).

Quantitative Determination of Sulfanilamide and Sulfathiazole in Mixtures

Spectrophotometric Method

D. T. ENGLIS AND DOUGLAS A. SKOOG[1], Noyes Chemical Laboratory, University of Illinois, Urbana, Ill.

THE wide use of the sulfa drugs has given rise to considerable interest in their analytical reactions. Their assay, differentiation, and identification have been the subject of a recent article by Calamari, Hubata, and Roth (*3*). The principal chemical reaction employed for the quantitative estimation of those substances involves the diazotization of the aryl amino group. By coupling the diazonium salt and production of an azo dye the compound may be estimated colorimetrically and the sensitivity greatly increased (*5*). Anderson (*1*) has called attention to the fact that free sulfanilamide and acetylated sulfanilamide have different absorption characteristics in the range 310 to 360 mμ and has devised a unique fluorometric method for the estimation of each.

A problem which was presented to the authors was the determination of sulfanilamide and sulfathiazole when present together in a mixture. It seemed probable that these compounds would show markedly different absorption characteristics in the ultraviolet range and that the determination of each could be accomplished by a physical method without separation of the constituents. In 1930 Barnard and MacMichael (*2*) demonstrated that a color system of two components may be quantitatively analyzed, even if both components show absorption at the selected wave length, provided the degree of absorption is sufficiently different and the sample represents a definite total amount of the two constituents only. Knudson, Meloche, and Juday (*4*) have pointed out an extension of the general principle and have indicated how the method may be used for more than two components. It is necessary to determine the extinction coefficients of each pure component at selected wave lengths which give the widest differences in absorption of one from the other components. The extinction value of the mixture is then determined at the selected wave lengths and the proportions of the various constituents are calculated from the data by the solution of a series of simultaneous equations.

It was the object of this study to develop a method for the analysis of the sulfa drug mixture based upon this principle.

Experimental

The instrument employed in this work was a Bausch & Lomb medium quartz spectrograph. The slit was adjusted to a width of 0.06 mm. A Wood's type hydrogen discharge tube, which gives a continuous spectrum of fairly uniform intensity over the desired wave-length range, served as the source of illumination. The source was placed with the exit window at a distance of 10 cm. from the slit. A cell of 1-cm. length with quartz windows was used to hold the liquids during their examination. Separate exposures of the solvent and the solutions were taken for a period

TABLE I. DETERMINATION OF SULFATHIAZOLE AND SULFANILAMIDE IN MIXTURES BY SPECTROPHOTOMETRIC EXAMINATION AT 2600 AND 2875 Å.

(Values expressed in mg. of constituent per liter)

	Sulfanilamide			Sulfathiazole		
Mixture	Added	Found	Deviation	Added	Found	Deviation
1	2.50	2.55	+0.05	1.25	1.17	−0.08
2	8.33	8.37	+0.04	1.60	1.42	−0.18
3	10.00	9.70	−0.30	2.00	1.97	−0.03
4	2.00	1.97	−0.03	10.00	10.40	+0.40
5	5.00	5.20	+0.20	5.00	5.09	+0.09
6	7.00	6.95	−0.05	3.00	3.11	+0.11
7	3.00	3.30	+0.30	7.00	7.50	+0.50
8	1.25	1.29	+0.04	2.50	2.70	+0.20

[1] Present address, Standard Oil Co., Richmond, Calif.

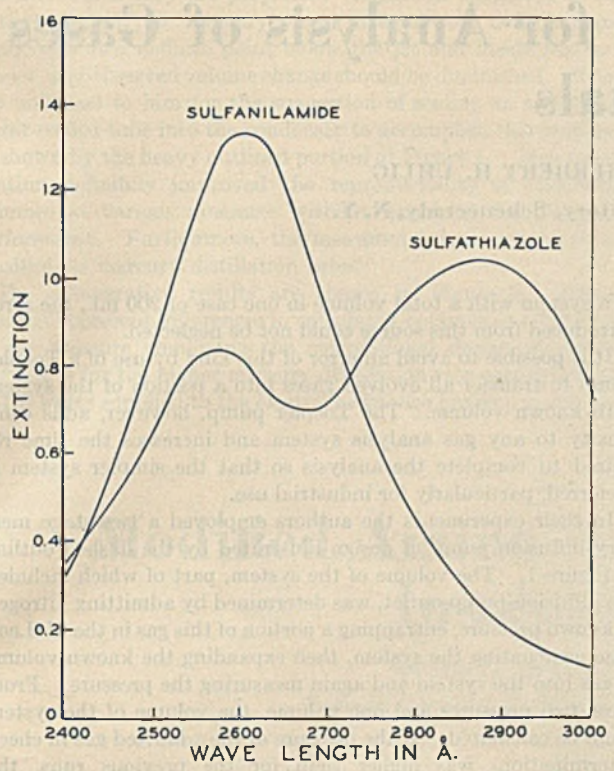


FIGURE 1. ABSORPTION CURVES

of 2 minutes each. The spectra were recorded on Eastman Polychromatic plates. Each plate was calibrated by taking a series of separate successive exposures in which the time interval was varied in a regular manner as follows: 4, 8, 16, 32, 64, and 128 seconds. The plates were developed for 6 minutes in Eastman x-ray developer at 18° C., then fixed, washed, and dried. After drying, the densities of the spectrograms at selected wavelength intervals were determined with a Leeds & Northrup recording microphotometer. A family of plate calibration curves for the selected wave lengths was then constructed and by reference to the appropriate curve the relative intensity values for the pure solvent and the solution were found and from these the extinction value for the solution was calculated.

Figure 1 shows the absorption curves for pure sulfathiazole and pure sulfanilamide in 95 per cent ethanol solutions. The sulfanilamide solution shows an absorption peak at 2610 Å. and the sulfathiazole solution has two absorption peaks, one at 2580 Å. and one at 2875 Å. The sulfanilamide has a slight absorption at 2875 Å. also.

The absorption values at different concentrations of each compound were determined and the results are indicated in Figure 2. The solutions of both compounds obey the Beer-Lambert law at both 2600 and 2875 Å. Therefore, a quantitative estimation of each of the constituents is possible in a mixture of the two.

The data necessary for making a calculation of the quantity of each are given below:

E'_a = extinction for 1 gram per liter of sulfathiazole at 2600 Å.
E'_b = extinction for 1 gram per liter of sulfanilamide at 2600 Å.
E''_a = extinction for 1 gram per liter of sulfathiazole at 2875 Å.
E''_b = extinction for 1 gram per liter of sulfanilamide at 2875 Å.
E'_m = extinction for the mixture at 2600 A.
E_m = extinction for the mixture at 2875 Å.

If x = grams of sulfathiazole per liter
y = grams of sulfanilamide per liter

Then $xE'_a + yE'_b = E'_m$
$xE''_a + yE''_b = E''_m$

From a solution of these equations the number of grams of each constituent in the solution is determined. The results of a group of analyses are given in Table I.

Discussion

An examination of Table I shows the order of agreement which may be expected in a series of analyses in which the ratios and total amount of the two constituents are varied. The procedure employed involved the assumption of a source of illumination of constant intensity. This assumption was checked by making a series of exposures of the source in which the time was constant for each. The variation was within the range of other experimental errors.

It is obvious that if the observations were made with an instrument which did not require the photographic process—for example, the photoelectric spectrophotometer adapted to the ultraviolet range—the errors which are inherent in the photographic method might be eliminated and the process of analysis greatly simplified. It is possible that solutions of higher concentration might be handled with such equipment and the accuracy of the method improved.

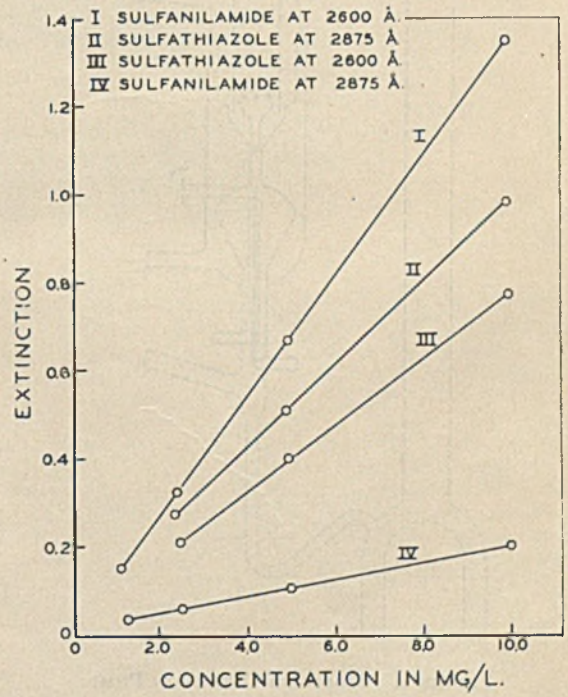


FIGURE 2. ABSORPTION VALUES

Acknowledgment

The authors wish to express their indebtedness to L. A. Hall The Griffith Laboratories, Chicago, Ill., at whose suggestion the problem was undertaken and from whom the samples employed in this study were obtained.

Literature Cited

(1) Anderson, S., IND. ENG. CHEM., ANAL. ED., 15, 29 (1943).
(2) Barnard, M., and MacMichael, P., *Ibid.*, 2, 363 (1930).
(3) Calamari, J. A., Hubata, R., and Roth, P. B., *Ibid.*, 14, 534 (1942).
(4) Knudson, H. W., Meloche, V. W., and Juday, C., *Ibid.*, 12, 715 (1940).
(5) Lee, S. W., Hannay, N. B., and Hand, W. C., *Ibid.*, 15, 403 (1943).

Modified Diffusion Pump for Analysis of Gases in Metals

JOHN J. NAUGHTON AND HERBERT H. UHLIG
General Electric Research Laboratory, Schenectady, N. Y.

IN RECENT years the effect of gases in metals on metal properties and the analyses of these gases have received increasing attention. The most direct methods for such analyses consist in heating or fusing the metal in a high vacuum and removing the evolved gases with the aid of a diffusion pump.

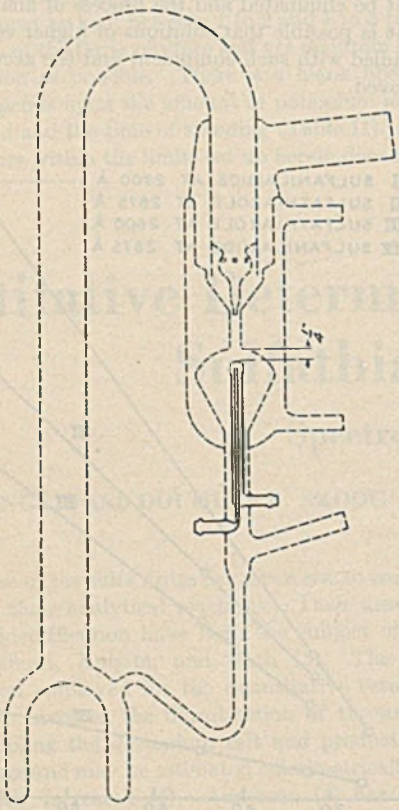


FIGURE 1. MODIFIED DIFFUSION PUMP

In certain schemes (*1*, *2*, *3*, *5*) employing a volumetric method of measuring gas quantities, the pump acts as a cutoff or valve to prevent return of evolved gases to the metal sample, and the outlet portion of the pump serves as part of the known volume which the gas occupies. It is usually assumed that the cutoff action of the mercury jet is sharp, and, consequently, that the volume of the system is constant for any gas pressure which is less than the critical back pressure under which the diffusion pump operates. The authors have observed, however, that the portion of the determined volume within the diffusion pump is to some extent dependent on gas pressure, and is, moreover, sensitive to the distillation rate of the mercury. The maximum variations observed were of the order of 35 ml. with major differences occurring in the low-pressure region (0 to 0.04 mm. of mercury). This maximum discrepancy may not be important if the total volume for gas confinement is of the order of liters, but in a system with a total volume in one case of 200 ml., the error introduced from this source could not be neglected.

It is possible to avoid an error of this kind by use of a Toepler pump to transfer all evolved gases into a portion of the system with known volume. The Toepler pump, however, adds complexity to any gas analysis system and increases the time required to complete the analysis so that the simpler system is preferred, particularly for industrial use.

In their experiments the authors employed a two-stage mercury diffusion pump of design illustrated by the dashed outline of Figure 1. The volume of the system, part of which included the diffusion-pump outlet, was determined by admitting nitrogen at known pressure, entrapping a portion of this gas in the McLeod gage, evacuating the system, then expanding the known volume of gas into the system and again measuring the pressure. From these two pressures and one volume, the volume of the system could be calculated. If the pressure of the admitted gas in check determinations was higher than for the previous runs, the calculated volume increased. In addition, if the mercury distillation rate was increased, the calculated volume decreased. These facts were checked by replacing the diffusion pump with another of the same general design and noting the same behavior.

The effect is not due to leakage past the mercury jet. Langmuir has shown (*4*) that pumps of this type will maintain a high vacuum against pressures greater than those used in the authors' determinations.

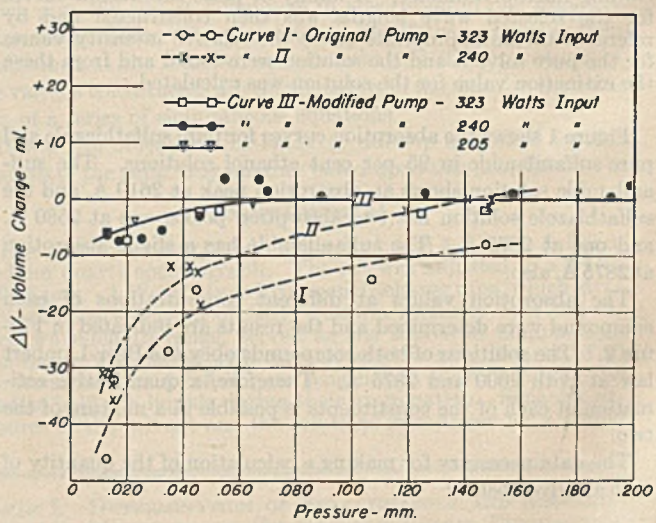


FIGURE 2. CHANGE OF CALCULATED VOLUME WITH PRESSURE AT VARIOUS DISTILLATION RATES

Apparently the change in volume with gas pressure is caused by the long mean-free-path mercury atoms traveling undeflected into lower portions of the pump condenser. They transform from the vapor phase at different levels, depending on the velocity of the mercury atoms and on the mean pressure of the

gas into which they are projected. Langmuir suggested to the authors that if the mercury vapor stream were deflected and condensed at a definite point below the jet and inside the condenser, any observed volume change should be diminished. They are indebted to him for the suggestion of sealing an additional water-cooled tube into the condenser to accomplish this purpose, as shown by the heavy outlined portion of Figure 1. This modification definitely improved the reproducibility of calculated volumes at various pressures without impairing the pumping performance. Furthermore, the measured volume was now insensitive to mercury distillation rates.

The comparative results are shown in Figure 2. Curves I and II represent differences in calculated volumes as a function of gas pressure employing the conventional design of pump. Curve I is for the higher mercury distillation rate corresponding to 323 watts supplied to the electric resistance heater; and curve II, the lower distillation rate, corresponding to 240 watts. Curve III is for a similar system employing the modified diffusion pump. Determined volumes of the system are better reproducible and the maximum deviation of the mean volume as the pressure is lowered to 0.01 mm. of mercury is less than 10 ml. Of further importance is the fact that the data at three distillation rates practically coincide.

Literature Cited

(1) Chipman, J., and Fontana, M. G., Ind. Eng. Chem., Anal. Ed., 7, 391 (1935).
(2) Derge, G., *Metals Tech.*, 10, T. P. 1544 (1943).
(3) Holm, V. C. F., and Thompson, J. G., *Bur. Standards J. Research*, 26, 245 (1941).
(4) Langmuir, Irving, *Gen. Elec. Rev.*, 19, 1060 (1916).
(5) Vacher, H. C., and Jordan, L., *Bur. Standards J. Research*, 7, 375 (1931).

Modified Amino Nitrogen Apparatus for Insoluble Proteins

DAVID G. DOHERTY AND CLYDE L. OGG
Eastern Regional Research Laboratory, Philadelphia, Penna.

An auxiliary reaction chamber for use with a manometric Van Slyke apparatus is described. The apparatus enables one to determine easily the rate of liberation of amino nitrogen from insoluble proteins and thereby to determine their free amino nitrogen content.

SINCE the chemical modification of proteins frequently involves the free amino groups, it is advantageous to be able to determine the free amino nitrogen remaining after chemical treatment. Most of the protein derivatives are insoluble under the conditions of analysis, and some have a rather low free amino nitrogen content. The limitations of the Van Slyke method (*7*) are well known and have been summarized by Richardson (*5*). Its application to insoluble proteins has offered additional difficulties in the introduction of the sample into the reaction chamber and the selection of the time of reaction. Several means for solving these difficulties have been suggested (*1, 2, 3, 6*). Kanagy and Harris (*1*) have shown that an arbitrary reaction time may not give the correct value for free amino nitrogen in proteins and have utilized the rate of evolution of amino nitrogen to determine the free amino nitrogen present. Rutherford, Harris, and Smith (*6*) using a modified Shepherd gas analysis apparatus extended this method.

It seemed worth while to use this principle with an apparatus that was simpler and more adapted to routine determinations. With these requirements in mind an accessory reaction chamber for use with a standard manometric Van Slyke apparatus was designed which will permit introduction of large samples of solid material, removal of the gas evolved at stated intervals, and easy cleaning of the chamber at the end of the analysis. The calibrated Van Slyke extraction chamber is used only for measurement of the nitrogen evolved. The amino nitrogen content of several proteins and substituted proteins was determined with the aid of the auxiliary chamber.

Apparatus and Reagents

Figures 1 and 2 show the details of the auxiliary chamber and its relation to the Van Slyke apparatus. The chamber was constructed from a 34/45 standard taper ground-glass joint and mounted on a plate fastened to the extraction chamber carrier of the Van Slyke apparatus. The outer top half was constricted and sealed to a three-way 1-mm. bore capillary stopcock, *A*, fitted with a cup and a bent side arm similar to the stopcock of the Van Slyke extraction chamber. The inner bottom half was also constricted and sealed to a piece of 5-mm. bore heavy-walled tubing long enough to reach the pivotal point of the extraction chamber carrier. A yoke was fastened to each half of the chamber, and two connecting springs were provided to keep the chamber together. A 5-mm. bore stopcock, *B*, was fastened to the base of the Van Slyke apparatus and connected to the chamber with a piece of heavy-walled tubing. A 250-cc. leveling bulb was connected to *B* through a length of heavy-walled nitrometer tubing and hung in one of two rings on an adjacent ring-stand. Sufficient mercury to fill the chamber and one third of the leveling bulb was added. The rings were arranged in such a manner that with the bulb in the lower ring the mercury level was slightly above the 5-mm. bore tube and with the bulb in the upper ring the level was 10 cm. above the top of the chamber.

The reagents used are a solution of 40 grams of sodium nitrite in 50 cc. of water, glacial acetic acid, a saturated solution of sodium acetate, and capryl alcohol. Rutherford, Harris, and Smith (*6*) have shown that addition of sodium acetate decreases the size of the blanks. The standard manometric Van Slyke–Hempel pipet is filled with a solution of alkaline permanganate consisting of 50 grams of potassium permanganate and 25 grams of potassium hydroxide made up to 1 liter with water. The ground-glass joint of the chamber is well lubricated with a stiff vacuum grease before being assembled. The samples used are all ground to 80-mesh in a Wiley micromill, and the moisture content is determined at the time of analysis. The amount of protein used ranges from 0.5 to 1 gram.

Experimental Procedure

An excellent description of the care and use of the manometric Van Slyke apparatus has been given by Peters and Van Slyke (*4*). One familiar with the standard manometric amino nitrogen determination may proceed with the modified method as follows:

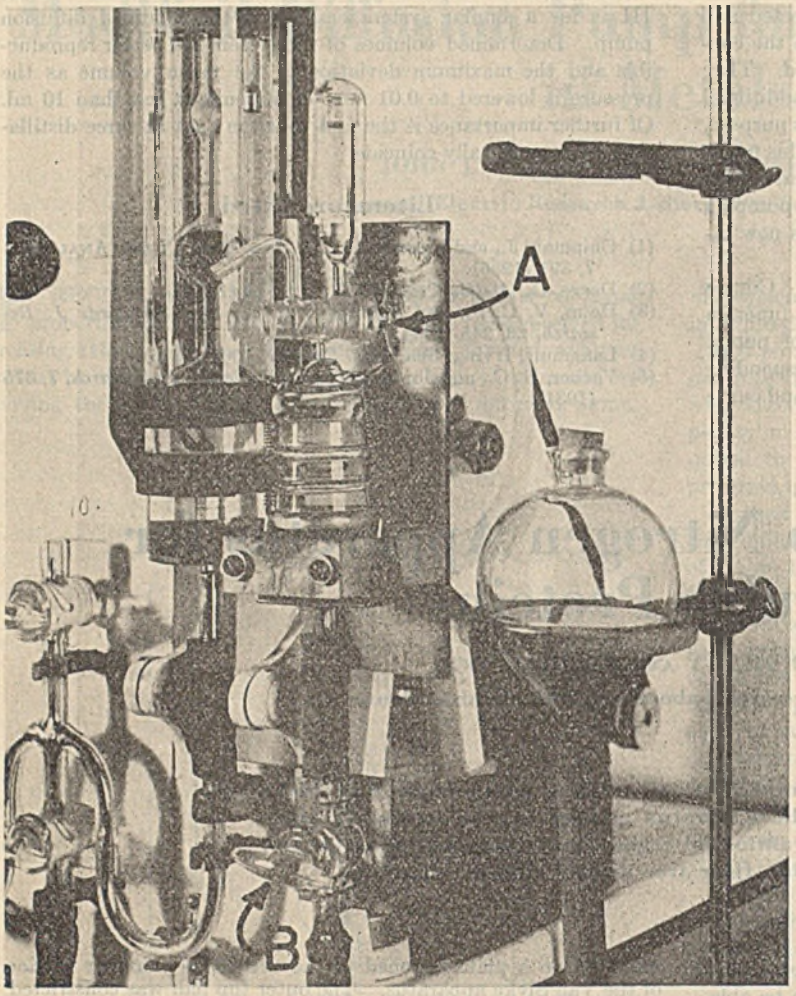


FIGURE 1. REACTION CHAMBER ATTACHED TO VAN SLYKE APPARATUS

Ten milliliters of water are admitted to the calibrated Van Slyke extraction chamber, the stopcock is closed, and the mercury is set at the 50-ml. mark. The top of the auxiliary chamber is removed, the mercury level set even with the bottom of the chamber, and stopcock *B* closed. The weighed sample of solid protein is introduced, followed by 2.0 ml. of glacial acetic acid, 2.0 ml. of saturated sodium acetate solution, and 5 drops of capryl alcohol. The chamber is assembled, and the bore of stopcock *A* and the capillary of the cup are filled with water. The side arm of *A* is connected with a high-vacuum pump, the pump started, and *A* turned to connect the pump with the chamber. The evacuation is continued for 2 minutes. The chamber is shaken throughout this period to facilitate removal of air from the mixture and prevent the formation of a cake. The shaking is then stopped, *A* is closed, and the vacuum line is disconnected.

Four milliliters of sodium nitrite solution are pipetted into the cup and run into the chamber by turning *A*, care being taken to admit no air. The cup and the bore of the stopcock are rinsed with 1 ml. of water, and the chamber is shaken 5 to 15 minutes, depending upon the amount of gas evolved. The shaking is then discontinued and *B* is opened to admit mercury to the chamber at an even rate. The leveling bulb is raised to the high ring to place the gas in the chamber under positive pressure. A few milliliters of water are placed in the cup, and the tip of a Hempel pipet is pressed in place. The Hempel stopcock is turned to connect the tip with the bulb, *B* is closed, and *A* is opened wide. *B* is then slowly opened, admitting mercury to the chamber and forcing the evolved gas into the Hempel pipet. *B* is closed when the reaction mixture is even with the bore of *A*. The Hempel stopcock is turned to the off position, the leveling bulb placed in the low ring, and *B* opened and then closed. The Hempel stopcock is turned to connect the cup with the tip, and *B* is opened slowly, allowing the gas in the Hempel capillary to be drawn back into the chamber along with enough water to seal the bore of *A*. *A* is closed, and the mercury level in the chamber is lowered until it is even with the bottom. *B* is then closed. The purified nitrogen in the Hempel pipet is transferred to the calibrated extraction chamber and measured in the usual manner.

Successive samples of gas are removed by the same procedure, the chamber being shaken for 10 minutes just prior to removal. The most effective control of liquids and gases is obtained through manipulation of the flow of the mercury through *B*.

Blank determinations were run in a similar manner, the protein being omitted. Selected time intervals generally were 10 minutes, 30 minutes, 1 hour, and each succeeding hour, the time being taken from the moment the sodium nitrite was added to the mixture. If the protein clings to the walls of the chamber when the mercury is lowered after a sample of gas has been removed, it may be dislodged by shaking the chamber while the mercury level is being lowered. The pressure readings obtained were corrected by subtracting a blank value for a similar time interval. The rate curves were obtained by plotting the total amino nitrogen liberated, in milligrams per gram of dry protein, against time. Duplicate values obtained from several proteins, modified proteins, arginine, and α-benzoylarginine amide by extrapolation of the secondary portion of the curve to zero time are given in Table I.

Discussion

Determinations were run in duplicate, and the rate curves for proteins and derivatives (Figure 3) plotted as previously described. The ratio of amino nitrogen to total nitrogen was plotted against time for arginine and α-benzoylarginine amide (Figure 4). The determinations were reproducible within the same limits of error as the manometric Van Slyke amino nitrogen determination (*4*). The values for the free amino nitrogen content of the proteins and derivatives in Table I were found by extrapolation of the secondary portion of the rate curve to zero time. This extrapolation is illustrated by the gelatin curve in Figure 3. The values can also be determined by calculation, using the straight-line equation:

$$y = mx + b$$

TABLE I. FREE AMINO NITROGEN CONTENT OF SOME PROTEINS AND DERIVATIVES DETERMINED AT 22–23° C.

Substance	Amino Nitrogen (Duplicate Determinations)	
	Mg./g. dry substance	
Wool	3.30	3.35
Silk (raw)	2.90	2.96
Gelatin (Eastman Kodak Co.)	8.70	8.74
Zein (Corn Products Refining Co.)	1.00	...
Casein	8.40	8.35
Benzoyl casein[a]	0.20	0.18
Desamino casein[a]	0.90	1.00
Arginine	80.2[b]	80.2
α-Benzoylarginine amide	0.0	0.0

[a] Prepared by J. L. Wood and A. P. Swain of this laboratory.
[b] Calculated, 80.4.

where y is the total milligrams of nitrogen per gram of dry protein obtained in time x, m the milligrams of nitrogen obtained per hour when the rate has become constant, and b the amount of free amino nitrogen initially present.

To achieve reproducible results and a rate curve capable of analysis by these methods, it is important to prevent the formation of aggregates in the chamber when the reagents are added. The material in the lumps so formed is not readily accessible to the action of the reagents and consequently evolves nitrogen slowly. The rate curve plotted from the values obtained in this instance has no definite change in slope and cannot be extrapolated in the usual manner to obtain an amino nitrogen value. This factor of aggregation is also of importance when the solution of a protein which is precipitated under the conditions of the analysis is admitted into the chamber. Thus the curd formed by an alkaline casein solution gave an indecisive rate curve similar to those obtained when aggregates of solid material were present. The use of a finely divided casein, well shaken to prevent the formation of lumps, obviated this difficulty. An examination of the curves obtained for the various proteins shows that one cannot arbitrarily select any reaction time that will be valid for different substances and conditions of analysis. Once the technique of handling this modified apparatus is acquired, the time required for the removal and measurement of a sample of gas—about 5 minutes—is approximately the same as in the standard Van Slyke determination. Since the reaction chamber is easily accessible and can be taken apart, it can be quickly and thoroughly cleaned at the end of each determination.

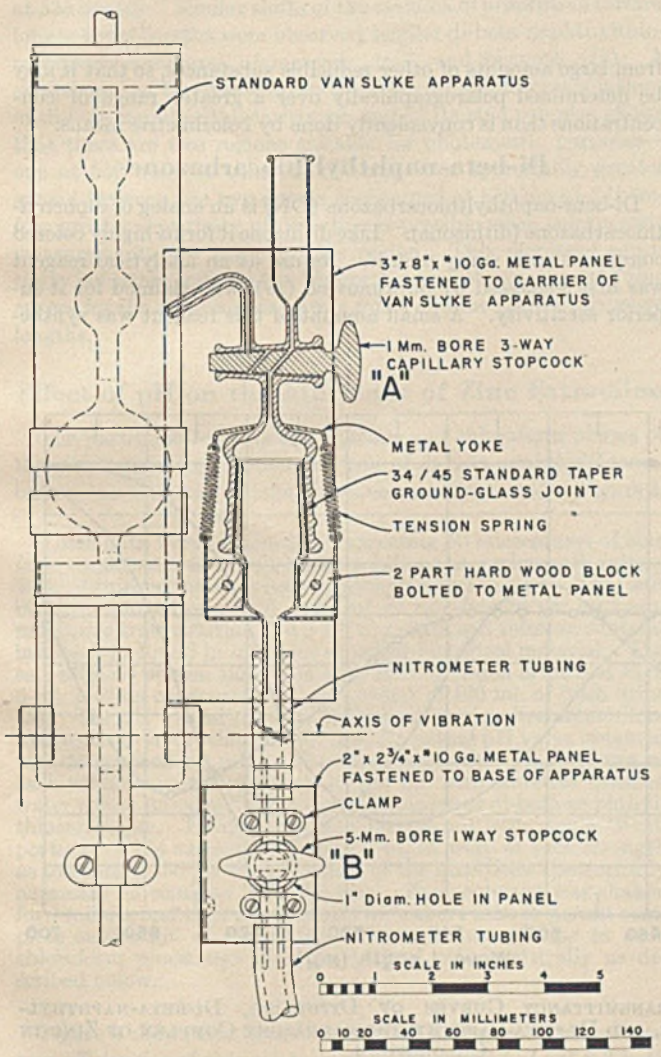


FIGURE 2. DIAGRAM OF REACTION CHAMBER

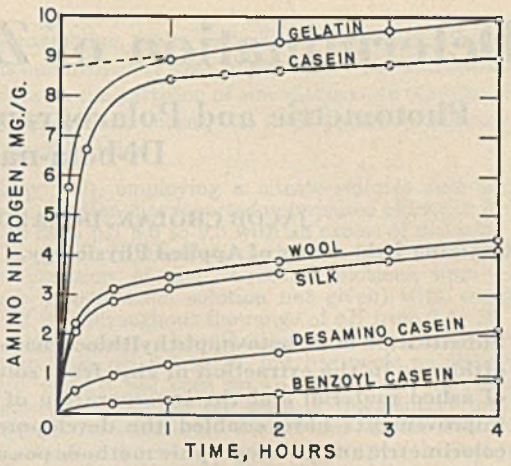


FIGURE 3. RATES OF EVOLUTION OF NITROGEN FROM VARIOUS PROTEINS

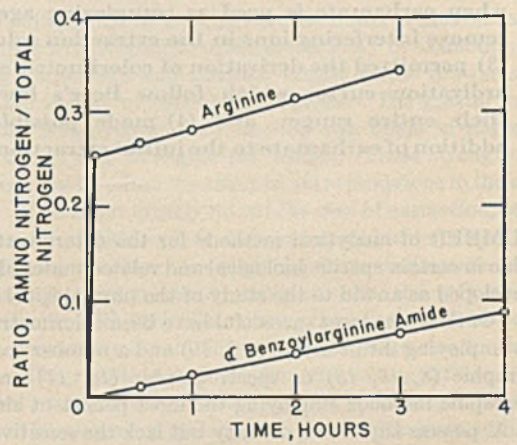


FIGURE 4. RATES OF EVOLUTION OF NITROGEN FROM ARGININE AND α-BENZOYLARGININE AMIDE

Summary

A simple apparatus for the routine determination of amino nitrogen of insoluble proteins and protein derivatives has been described. The rate of evolution of amino nitrogen from some insoluble proteins and protein derivatives was determined with this apparatus. The curves obtained by plotting the amino nitrogen content against time are similar to those obtained by Rutherford, Harris, and Smith (6) using a modified Shepherd gas analysis apparatus.

Literature Cited

(1) Kanagy, J. R., and Harris, M., *J. Research Natl. Bur. Standards*, 14, 563 (1935); *Research Paper* 787.
(2) Klemm, O., and Meyer, K. H., *Helv. Chim. Acta*, 23, 1444 (1940).
(3) Lieben, F., and Loo, Y. C., *J. Biol. Chem.*, 145, 223 (1942).
(4) Peters, J. P., and Van Slyke, D. D., "Quantitative Clinical Chemistry", Vol. II (Methods), p. 385, Baltimore, Williams & Wilkins Co., 1932.
(5) Richardson, G. M., *Proc. Roy. Soc.*, Ser. B115, 142 (1934).
(6) Rutherford, H. A., Harris, M., and Smith, A. L., *J. Research Natl. Bur. Standards*, 19, 467 (1937); *Research Paper* 1038.
(7) Van Slyke, D. D., *J. Biol. Chem.*, 9, 185 (1911).

Determination of Zinc in Biological Material

Photometric and Polarographic Methods Following Extraction with Di-beta-naphthylthiocarbazone

JACOB CHOLAK, DONALD M. HUBBARD, AND ROLAND E. BURKEY
Kettering Laboratory of Applied Physiology, College of Medicine, University of Cincinnati, Cincinnati, Ohio

Substitution of di-beta-naphthylthiocarbazone for dithizone in the extraction of zinc from solutions of ashed material and the incorporation of other improvements have enabled the development of colorimetric and polarographic methods possessing excellent sensitivity and specificity. The substitution of di-beta-naphthylthiocarbazone for dithizone has (1) eliminated partition losses occurring with dithizone in the pH range 8.3 to 10.5; (2) eliminated the loss of zinc which occurs otherwise when carbamate is used as complexing agent to remove interfering ions in the extraction solution; (3) permitted the derivation of colorimetric standardization curves which follow Beer's law over their entire range; and (4) made possible the addition of carbamate to the initial extraction step, a procedure which allows the analyst to fix the range and to extract zinc in sufficient purity to be determined either colorimetrically or polarographically.

Substitution of 0.2 *N* for 0.02 *N* hydrochloric acid permits stripping at least 60 micrograms of zinc from di-beta-naphthylthiocarbazone–carbamate-chloroform solution with a single 50-ml. portion of acid while eliminating interference by copper, cobalt, nickel, mercury, and silver.

The colorimetric method of estimation is recommended for determining very small quantities of zinc in samples containing no cadmium. When cadmium is present or when the quantities of zinc are greater than 0.05 mg. per 10 ml. of solution, the polarographic method is recommended.

A NUMBER of analytical methods for the determination of zinc in certain specific biological and related materials have been developed as an aid to the study of the physiological effects of zinc. Of these the most successful have been colorimetric procedures employing dithizone (*6*, *7*, *9*, *10*) and a number based on polarographic (*8*, *16*, *18*) or spectrographic (*15*, *17*) analysis. Spectrographic methods employing the most persistent zinc line at 2138 A. possess superior specificity but lack the sensitivity required for general biological work (*5*). Polarographic methods are satisfactory in respect to sensitivity, but high concentrations of many substances which are reduced at potentials below or near that of zinc may affect the polarographic wave of zinc. Colorimetric methods employing dithizone are extremely sensitive but require careful control of pH and prevention of the interferences of certain ions by the formation of nonreactive complexes. Thus far no reagent has been found which, when used with dithizone, will form satisfactory complexes with interfering ions without causing some loss of zinc. Through the use of these colorimetric methods, therefore, reproducible results can be obtained only by a rigid control of conditions to ensure uniform losses or contaminations.

In the methods described in this paper losses of zinc in extraction and most of the difficulties due to interfering ions are eliminated by employing a chloroform solution of di-beta-naphthylthiocarbazone and aqueous sodium diethyldithiocarbamate as the extraction agent. By the use of this extraction agent and the introduction of a number of other improvements, a sensitive and accurate mixed-color method has been developed. The initial extraction in the colorimetric procedure provides a means of concentrating the zinc and of removing it from large amounts of other reducible substances, so that it may be determined polarographically over a greater range of concentrations than is conveniently done by colorimetric means.

Di-beta-naphthylthiocarbazone

Di-beta-naphthylthiocarbazone (DN) is an analog of diphenylthiocarbazone (dithizone). Like dithizone it forms highly colored complexes with many metals. Its use as an analytical reagent was first suggested by Suprunovich (*19*), who claimed for it superior sensitivity. A small amount of this reagent was synthe-

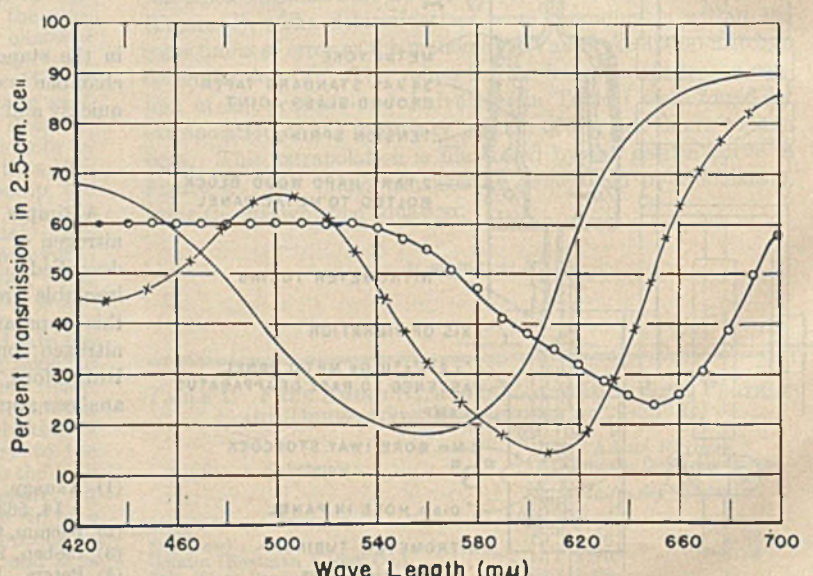


FIGURE 1. TRANSMITTANCY CURVES OF DITHIZONE, DI-BETA-NAPHTHYLTHIOCARBAZONE, AND DI-BETA-NAPHTHYLTHIOCARBAZONE COMPLEX OF ZINC IN CHLOROFORM

o. Pure DN in chloroform, 2 mg. per liter
x. Pure dithizone in chloroform, 2 mg. per liter
–. Pure DN totally converted to zinc complex

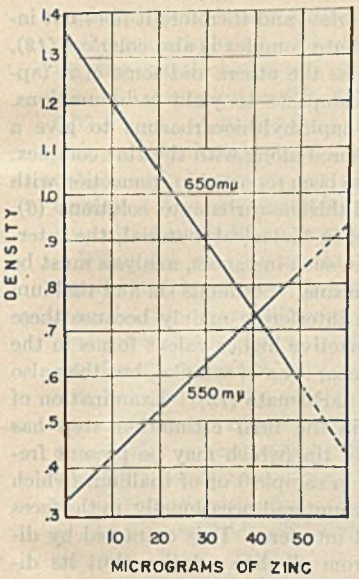


FIGURE 2. STANDARD CURVES FOR ESTIMATION OF ZINC
2.5-cm. cell

sized by Hubbard and used for the determination of mercury in urine (*13*). Since that time an improved method of synthesis has been developed whereby the reagent can be produced in greater purity and in a yield sufficient to facilitate more complete study of its properties. This method will be described in another paper (*14*).

The transmittancy curves for chloroform solutions of dithizone, of di-beta-naphthylthiocarbazone, and of the pure zinc–di-beta-naphthylthiocarbazone complex are shown in Figure 1. Absorption of the pure zinc complex is greatest at 550 mμ, in contrast to zinc dithizonate which has its maximal absorption at 535 mμ (*6*). Similar shifts of the maxima of absorption toward longer wave lengths were observed for the di-beta-naphthylthiocarbazone complexes of mercury, lead, and bismuth (*13*). A comparison of the transmittancy curves for chloroform solutions of di-beta-naphthylthiocarbazone and of its zinc complex shows that there are two regions suitable for photometric purposes—one at 550 mμ, the other at 650 mμ. An appreciably greater spread between the two curves is obtained at 650 mμ and therefore greater sensitivity of detection is possible when colorimetric measurements are made in this region. This increased sensitivity is further indicated in Figure 2, which shows standard zinc curves for the same concentration range obtained at the two wave lengths.

Effect of pH on the Efficiency of Zinc Extraction

The partitions between the aqueous and chloroform phases of the zinc complexes of dithizone and of di-beta-naphthylthiocarbazone, occurring at various pH values, are illustrated in Figure 3.

These data were obtained by extracting 50 micrograms of zinc from duplicate sets of salt buffer solutions at various pH values, with dithizone and di-beta-naphthylthiocarbazone. The salt buffer employed consisted of 30 ml. of zinc-free 20 per cent ammonium citrate solution and 5 ml. of a stock salt solution containing the ions found in solutions of ashed biological material. The salt solution chosen simulated a solution of ashed urine and each 5-ml. portion contained the ions present in 100 ml. of fresh urine (*3*). The pH was adjusted by adding either zinc-free ammonium hydroxide or hydrochloric acid, and the actual pH value obtained was determined by means of a glass electrode. The volume of each solution was brought up to 100 ml. with zinc-free distilled water before its extraction with the dithizone or di-beta-naphthylthiocarbazone. Extractions were carried out with single 10-ml. portions of the extraction reagents, which were of such strength as to contain 150 per cent in excess of the quantities theoretically necessary to combine with the zinc. Each solution was shaken for 1 minute and then was set aside for a short time to permit complete separation of the phases. The amount of zinc in each chloroform phase was then determined colorimetrically as described below.

Figure 3 shows that there is a decided difference in the extraction efficiencies of the two reagents. At a value of pH 8.3 both reagents extract zinc quantitatively. As the pH values increase above pH 8.5 there is a marked drop in the efficiency of the dithizone extraction, while that of di-beta-naphthylthiocarbazone remains unimpaired at least to pH 10.5. The literature contains a few data on the partition of zinc dithizonate between the aqueous and chloroform phases, but these are not in agreement with the authors' findings.

Walkley (*20*), employing a citrate–sulfuric acid–ammonium hydroxide solution, has reported unimpaired efficiency for a range of values from pH 8.0 to 9.5 with an excess of dithizone of only 37 per cent. In Hibbard's investigation (*10*) of the effect of pH on the partition of zinc, aqueous solutions were extracted (strength of extraction solution not given) with complete recovery of zinc throughout the range of pH from 7 to 10. Wichmann (*21*) has given a hypothetical curve which shows complete extraction from pH 6.5 to 10, but has made no claim as to its accuracy, since it had been drawn so as to be analogous to the lead extraction curve on the basis of fragmentary information gleaned from the literature. Biefeld and Patrick (*2*) have shown that other anions have a profound effect upon the lead partition in dithizone extraction and that this partition is also affected by the strength of the dithizone solution employed. Both of these factors account for the discrepancies between the authors' results and those of Walkley (*20*) and Hibbard (*10*). The latter investigators did not take into account all of the ions present in prepared biological specimens.

The authors' use of the salt solution buffer creates satisfactory conditions with respect to uniform efficiency of extraction for obtaining comparable and accurate results in the routine analysis of a wide variety of biological materials. The greater excess of dithizone employed in their method also tends to improve the extraction of zinc at higher pH values. These strong solutions are necessary to offset the effect of extraneous ions in the aqueous phase. The latter greatly retard the rate of extraction, so that a large excess of dithizone or di-beta-naphthylthiocarbazone must be employed to convert the zinc to the zinc dithizonate or the di-beta-naphthylthiocarbazone complex. As the pH value increases, more dithizone dissolves in the alkaline phase and this causes an increased solubility of zinc dithizonate in the same phase. The result is that the zinc and excess dithizone can be removed completely from alkaline media above pH 8.0 only by repeated treatment of the aqueous phase with fresh additions of dithizone followed by repeated washing with pure chloroform.

The insolubility of di-beta-naphthylthiocarbazone in alkaline aqueous solutions eliminates the necessity for critical control of the pH and reduces the manipulations required to extract the

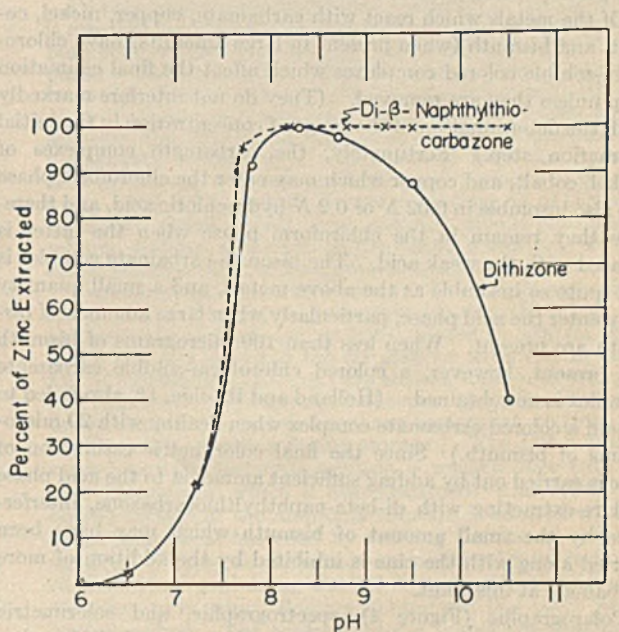


FIGURE 3. EFFECT OF pH ON ZINC PARTITIONS WHEN DITHIZONE AND DI-BETA-NAPHTHYLTHIOCARBAZONE EXTRACTIONS ARE MADE

zinc. The use of the reagent also limits the opportunities for loss of zinc to the mechanical factor of entrainment of droplets of the chloroform phase in the solution being tested. The small amount of the zinc–di-beta-naphthylthiocarbazone complex so held in the test solution may be reclaimed by a single treatment with pure chloroform or by the addition of a fresh portion of di-beta-naphthylthiocarbazone reagent following the addition of the last portion of di-beta-naphthylthiocarbazone which has retained its original color. The fact that di-beta-naphthylthiocarbazone is insoluble, however, makes it impossible to remove the excess of the reagent and therefore only "mixed color" colorimetric methods are applicable when di-beta-naphthylthiocarbazone is used as the extraction agent.

Elimination of Interferences

Chloroform solutions of di-beta-naphthylthiocarbazone extract the same metals as dithizone at pH 8 and therefore other metals beside zinc must be prevented from forming colored complexes. Sodium diethyldithiocarbamate added to the aqueous phase has been shown to be effective in preventing the formation of dithizonates of all metals but zinc (*6*, *12*). However, in the case of dithizone, carbamate prevents the complete extraction of zinc, the addition of as little as 12.5 mg. to the final estimation step resulting in the recovery of only 84 per cent of the zinc known to be present (*6*). This loss, added to that due to partition of zinc dithizonate in the final estimation step, results in standardization curves which do not obey Beer's law for the entire range of 0 to 30 micrograms of zinc (*6*). Therefore, when dithizone is used, reproducible results can be obtained only by strict control of the conditions of the procedure in handling the samples and standards, in order to maintain comparable losses of zinc in both.

The use of di-beta-naphthylthiocarbazone, on the other hand, results in a marked improvement in the extraction of zinc, the extraction being complete in the presence of even 50 mg. of carbamate. Standardization curves obtained with this reagent show no deviation from Beer's law for a range of values from 0 to 60 micrograms of zinc (Figure 2). This permits the addition of carbamate in the initial extraction step as well as in the final estimation step. The use of carbamate with di-beta-naphthylthiocarbazone in this fashion enables the analyst to fix the concentration range in the initial extraction step, a procedure which was not possible when dithizone was used.

Of the metals which react with carbamate, copper, nickel, cobalt, and bismuth (when present in large amounts) have chloroform-soluble colored complexes which affect the final estimation step unless they are removed. (They do not interfere markedly with the determination of the range of concentration in the initial extraction step.) Fortunately, the carbamate complexes of nickel, cobalt, and copper which may enter the chloroform phase are also insoluble in 0.02 *N* or 0.2 *N* hydrochloric acid, and therefore they remain in the chloroform phase when the latter is treated with the weak acid. The bismuth-carbamate complex is not quite so insoluble as the above metals, and a small quantity may enter the acid phase, particularly when large amounts of bismuth are present. When less than 100 micrograms of bismuth are present, however, a colored chloroform-soluble carbamate complex is not obtained. (Holland and Ritchie, *12*, also failed to obtain a colored carbamate complex when dealing with 20 micrograms of bismuth.) Since the final colorimetric estimation of zinc is carried out by adding sufficient ammonia to the acid phase and re-extracting with di-beta-naphthylthiocarbazone, interference by the small amount of bismuth which may have been carried along with the zinc is inhibited by the addition of more carbamate at this point.

Polarographic (Figure 4), spectrographic, and colorimetric studies of the acid wash of the initial di-beta-naphthylthiocarbazone extraction fail to show the presence of copper, nickel, cobalt, and mercury, but show that much of the lead and cadmium and a small amount of bismuth are carried along with the zinc. The lead-carbamate complex is colorless and therefore it does not interfere. The cadmium-carbamate complex is also colorless (*12*), but is evidently not so stable as the others and some of it (approximately 10 per cent) decomposes to yield cadmium ions. These combine with di-beta-naphthylthiocarbazone to give a colored complex which is measured along with the zinc complex. This action of cadmium has also been reported in connection with the extraction of zinc by dithizone-carbamate solutions (*6*). Since cadmium is rarely found in biological material, the interference will occur rarely, and in such instances, analysis must be carried out by polarographic means. Stannous tin and thallium (thallous) are prevented from interfering mainly because these metals are converted to the inactive higher valent forms in the procedures employed in the preparation of samples, but they also form colorless complexes with carbamate (*12*). Examination of spectra of the solution used in the final estimation step has failed to reveal the presence of tin (which may be present frequently and in large amounts in samples) or of thallium (which is rarely present). Silver, encountered occasionally, in the feces and in cooked foods, does not interfere. It is extracted by di-beta-naphthylthiocarbazone from alkaline solution, but its di-beta-naphthylthiocarbazone complex is stable in weak acid and therefore remains in the chloroform phase when the latter is shaken with 0.2 *N* hydrochloric acid. In addition, it forms a colorless complex with carbamate, so that any silver which may enter the final estimation solution in this form does not interfere.

In analyzing small volumes of solutions of prepared material of high ash content, some difficulty was encountered in extracting zinc unless the salt concentration was reduced by dilution with zinc-free water. For this reason it became a standard procedure to dilute the aliquot to 100 ml. with water before extraction.

It was also found that a single treatment of the initial di-beta-naphthylthiocarbazone extraction solution with 50 ml. of 0.02 *N* hydrochloric acid was insufficient to strip the zinc completely from the chloroform phase. Two factors were responsible for this—the presence of extraneous salts in the initial di-beta-

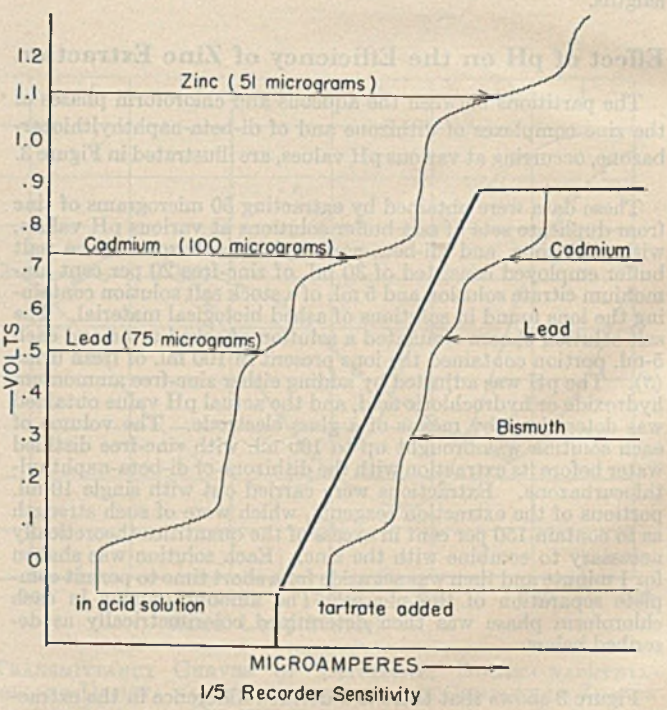


Figure 4. Polarograms Showing Effects of Certain Ions on Zinc Estimation

naphthylthiocarbazone extraction solution and the low strength of the acid employed. The first factor was eliminated by washing the initial chloroform phase with zinc-free water, and the second by employing 0.2 *N* hydrochloric acid in place of the 0.02 *N* acid. A single 50-ml. portion of the 0.2 *N* acid completely stripped 60 micrograms of zinc and yet failed to extract copper, cobalt, nickel, mercury, or silver. When the 0.02 *N* acid was employed it was not possible to strip more than 30 micrograms of zinc with a single 50-ml. portion of the acid.

When the zinc estimation is made polarographically, substances which reduce at potentials near that of zinc must be removed. This is true of nickel and cobalt, the waves of which may coalesce with the zinc wave if their concentration is high. The interference, therefore, is of a quantitative rather than a qualitative nature and any substance that will give a polarographic wave below, as well as near that of zinc, will also interfere if present in high concentration. Figure 4 shows a number of these possible interferences. Here it may also be seen that at high recorder sensitivities the amounts of these reducible substances must be diminished sufficiently to permit the zinc wave to appear on the chart. The initial di-beta-napthylthiocarbazone extraction as described below satisfactorily removes the zinc from iron, copper, nickel, cobalt, aluminum, phosphate, sulfate, and most of the bismuth and tends to reduce the quantities of lead and cadmium which are shaken into the acid phase employed for polarographic estimation. The extraction serves also to concentrate the zinc into small volume, thereby permitting greater accuracy in the analysis of materials low in zinc.

Procedures

Preparation of Samples. Samples are prepared for analysis by a combination of digestion and ashing procedures. The sample, consisting of weighed tissue or other material (10 to 20 grams) or 100 ml. of urine, is placed in a 200-ml. glazed silica evaporating dish. (When not in use the dishes are immersed in dilute nitric acid—1 part of nitric acid, sp. gr. 1.42, to 1 part of distilled water—in order to remove surface contamination.) Ten milliliters of redistilled nitric acid are added and the sample is taken to dryness on a hot plate. Organic matter is destroyed by igniting the sample in an electric muffle furnace maintained at 500° C. The complete destruction of organic matter may be hastened if the ash is treated with a little nitric acid from time to time, evaporated to dryness, and replaced in the muffle furnace. The clean ash is taken up in a little zinc-free hydrochloric acid and triple-distilled water. Insoluble residues are not filtered off but are carried along with the solution through the extraction steps. As little nitric acid should be employed as is consistent with rapid ashing, since it is not possible to free this reagent of zinc by distillation prior to its use. A blank analysis must be run with each series of samples, therefore, in order to correct for the zinc in the reagents.

Reagents. *Zinc-free distilled water* is obtained by redistilling double-distilled water in a Pyrex still.

Zinc-free ammonium hydroxide. Reagent ammonium hydroxide is distilled into triple-distilled water which is chilled in an ice bath.

Zinc-free hydrochloric acid is prepared by dropping a volume of concentrated sulfuric acid into an equal volume of concentrated hydrochloric acid in a Pyrex flask. A separatory funnel, its stem extending to a point just below the surface of the hydrochloric acid, is used for this purpose. The hydrogen chloride which is evolved is carried into the water by means of a delivery tube extending to a point just below the surface of the triple-distilled water kept in an ice bath. The strength of the zinc-free hydrochloric acid is obtained from standard tables, on the basis of the specific gravity as determined with a Westphal balance. From this acid, 0.2 *N* hydrochloric acid is made by dilution with triple-distilled water.

40 per cent ammonium citrate solution. Four hundred grams of citric acid are dissolved in water and sufficient ammonium hydroxide is added to make the solution just alkaline to thymol blue. The solution is made up to 1 liter with distilled water. Before use, the quantity required for 1 day is placed in a large separatory funnel, and is diluted with an equal volume of water. This 20 per cent solution is then shaken with a di-beta-napbthylthiocarbazone chloroform solution until the di-beta-naphthylthiocarbazone retains its original color.

Carbamate solution. One and a quarter grams of sodium diethyldithiocarbamate are dissolved in 100 ml. of triple-distilled water; 1 ml. = 12.5 mg. of carbamate. (This solution must be made up fresh daily.)

Extraction and standard di-beta-naphthylthiocarbazone solutions. DN solution 1 (0 to 5 microgram range). Twenty milligrams are dissolved in 1 liter of redistilled chloroform containing 10 ml. of absolute ethyl alcohol.

DN solution 2 (0 to 50 microgram range). Two hundred milligrams are dissolved in 1 liter of redistilled chloroform containing 10 ml. of absolute ethyl alcohol. (Both solutions are stored in brown bottles and are kept in the refrigerator when not in use.)

Chloroform Recovery. Used di-beta-naphthylthiocarbazone solutions are collected in glass-stoppered amber bottles and the chloroform is reclaimed in the manner described by Bambach and Burkey (*1*).

Apparatus. Pyrex ware is used throughout. Before use, extraction funnels should be rinsed with dilute nitric acid, followed by several rinsings with distilled water. Squibb-type separatory funnels are employed. These are of 150-ml. capacity with graduations at 5-, 10-, and all subsequent 10-ml. intervals up to 100 ml.

The densities of the colored solutions used in the zinc estimation were obtained with a photoelectric spectrophotometer, but any photometer, photoelectric or visual, which is equipped with a suitable filter and with 1-cm. and 2.5-cm. cells, may be employed. Filters with maximum transmission at either 550 or 650 mμ are preferred.

Polarographic estimations were made with the Leeds & Northrup Electro-Chemograph.

Initial Extraction Step. The entire sample or a suitable aliquot is placed in a 150-ml. separatory funnel. To this are added 30 ml. of 20 per cent ammonium citrate solution and 4 drops of 0.1 per cent aqueous thymol blue, followed by zinc-free ammonium hydroxide (sp. gr. 0.9) until pH 9.5 is reached. Four milliliters of carbamate solution (50 mg.) are now added and zinc-free water to the 100-ml. mark, and the solution is shaken for 1 minute with 5 ml. of extraction DN solution 1. [This will show whether the amount of zinc is less than 5 micrograms and permit the placing of the range. The color obtained is a faint violet (deeper shades of red indicate more than 5 micrograms) but some experience is necessary to recognize the range definitely.] Five milliliters of extraction DN solution 2 are added and the mixture is again shaken for 1 minute. If the range exceeds 5 micrograms, extractions with 5-ml. portions of the stronger solution are repeated until the last portion retains its original bluish-green color, each portion being drained into a second funnel before the next is added. (One should judge the color while shaking the solution, since the differences in color are more readily discernible in the shaken mixture than in the small volume of the chloroform phase.) The collected chloroform extracts are now washed with a 50-ml. portion of triple-distilled water and are drained into another funnel.

Di-beta-naphthylthiocarbazone entrained in the aqueous phase is removed by shaking the latter with one or two 5-ml. portions of pure chloroform, which are also added to the washed chloroform phase. The chloroform solution (or an aliquot containing not more than 50 micrograms of zinc) is now shaken with 50 ml. of 0.2 *N* hydrochloric acid and after the phases have been allowed to separate, the chloroform phase is discarded. Entrained di-beta-naphthylthiocarbazone is then removed from the hydrochloric acid by one or two washings with 5-ml. portions of pure chloroform. The 0.2 *N* hydrochloric acid contains all of the zinc freed of copper, nickel, cobalt, iron, mercury, silver, phosphates, aluminum, sulfates, and most of the bismuth and may be used for either polarographic or colorimetric estimation of the zinc.

Colorimetric Estimation Step. In another separatory funnel 45 ml. of ammonium hydroxide solution [50 ml. of zinc-free ammonium hydroxide (sp. gr. 0.90) diluted to 1000 ml. with zinc-free water and stored in the refrigerator when not in use] and 1 ml. of carbamate (12.5 mg.) are shaken with 5 ml. of extraction DN solution 1. (This treatment serves as a precautionary measure to remove zinc which may have been taken up from the glass by the weak ammonia during storage.) The DN extract is discarded and the ammonia solution is washed once with 5 ml. of pure chloroform, which is also discarded. Chloroform floating on the surface of the ammonia solution is allowed to evaporate or its removal is hastened by swirling the funnel and applying suction. The weak carbamate-ammonia mixture is then added to the funnel containing the 0.2 *N* hydrochloric acid extract of the zinc, from which floating chloroform has been removed as just described. The contents of the funnel are then mixed and the solution is shaken for 1 minute with 10 ml. of the proper extraction DN solution (DN solution 2 for the 0 to 50 microgram range and DN solution 1 for the 0 to 5 microgram range).

Table I. Recoveries of Zinc Added to Synthetic Urine Ash (3)

(Samples correspond to 100 ml. of urine)

Zinc Added *Micrograms*	Zinc Found *Micrograms*	Zinc Recovered by Colorimetric Method *Micrograms*
0	2.2	....
0	2.2	....
1	3.0	0.8
1	3.2	1.0
5	7.0	4.8
5	7.0	4.8
30	33.0	30.8
30	32.0	29.8
50	52.0	49.8
50	52.0	49.8
500	500	498
500	490	488

Table II. Recoveries of Zinc

(In the presence of other metals which form DN complexes. Samples correspond to 100 ml. of urine)

Zinc Added, Micrograms	Other Metals Added, 100 Micrograms of Each	Zinc Recovered by Colorimetric Method, Micrograms
0	Hg, Pb, Cu, Bi	0
0	Hg, Pb, Cu, Bi	0
50	Hg, Pb, Cu, Bi	50
50	Hg, Pb, Cu, Bi	51 (polarograph 53)
50	Hg, Pb, Cu, Bi, Co, Cd	62 (polarograph 51)

For the estimation of 0 to 5 micrograms of zinc, 5 ml. of the DN extract are run quantitatively into a 25-ml. glass-stoppered cylinder and the volume is made up to 25 ml. with pure chloroform. A 2.5-cm. cell is then filled and the density of the solution is obtained at either 550 or 650 mμ.

When the 0 to 50 microgram range is used, 5 ml. of the DN extract are diluted to 100 ml. with chloroform and the density of the solution is obtained in a 1-cm. cell.

The amount of zinc present is then determined from standard curves (Figure 2) obtained by carrying known amounts of zinc in 0.2 *N* hydrochloric acid through the final colorimetric estimation step.

Polarographic Estimation. The 0.2 *N* hydrochloric acid extract obtained at the end of the initial extraction step is transferred to a beaker of suitable size and is then evaporated to a volume of 1 to 2 ml. After cooling, the acidity of the solution is adjusted until it is just acid to methyl red (zinc-free ammonia and 0.2 *N* hydrochloric acid) and the solution is transferred to a glass-stoppered 10-ml. Pyrex graduated cylinder, the volume being made up to 10 ml. with distilled water. A portion of this solution is placed in a polarograph cell, and after oxygen has been removed from the solution by bubbling tank nitrogen through it for 5 minutes, the polarogram is obtained from −0.8 to −1.3 volts. The zinc wave occurs at −1.1 volts (empirical, uncorrected) and its size is a measure of the concentration of the zinc in the solution. The quantity of zinc present in the 10-ml. volume is obtained by reading the value for the zinc step from a calibration curve obtained by treating known quantities of zinc in 0.2 *N* hydrochloric exactly as was done for the sample. Curves are derived for a number of recorder sensitivities at constant temperature and with a constant mercury drop rate. Recorder sensitivities of $^1/_5$, $^1/_{10}$, and $^1/_{40}$ will permit the determination of quantities of zinc varying from 5 micrograms to 1 or more milligrams per 10 ml. of solution.

Two other methods for evaluating polarograms may be used. The first method, employing an internal standard (4), has been found to be very accurate and eliminates the need for control of temperature, drop rate, or composition of the solution in the cell. In this method, 1 ml. of a cadmium solution (1 ml. = 0.06 mg.) is added to the beaker containing the residual of the 0.2 *N* acid evaporation. There is no change in the remainder of the procedure except that the polarograms are obtained from −0.5 to −1.3 volts. The ratio of the length of the zinc step to that of the cadmium (−0.72 volt empirical, uncorrected) is then read from calibration curves obtained by analyzing known amounts of zinc in the same manner. Obviously, the sample must be free from cadmium when this method is employed.

The other method, known as the standard addition method (11), is very convenient for use in the analysis of occasional samples, since it eliminates the need for the derivation and occasional checking of calibration curves. A polarogram is taken of a definite known volume of the test solution. After the addition of a known volume of a standard zinc solution, the polarogram is repeated and the concentration of the test solution is obtained from Hohn's formula (11):

$$\text{Concentration of unknown} = \frac{XvC_s}{x(V+v)+Xv}$$

where X = length of original step
x = increased length due to addition of the standard
C_s = concentration of standard solution
V = original volume of unknown in cell
v = volume of standard solution added

Reliability of the Methods

In Table I are recorded the recoveries obtained by the colorimetric procedure when known amounts of zinc were added to 5-ml. portions of a simulated urine salt stock solution (3) and the resultant samples were carried through the entire procedure, including the ashing step. The recoveries show no loss of zinc when samples are ignited at 500° C., nor does any loss result from the use of nitric acid as ashing agent. The blank of 2.2 micrograms shown in the table is due principally to the use of the nitric acid.

In Table II are listed recoveries for known amounts of zinc added to the stock urine salt solution (samples equivalent to 100 ml. of urine) which contained in addition 100 micrograms each of mercury, copper, lead, and bismuth. Also shown is a zinc recovery for a solution containing 100 micrograms each of mercury, bismuth, cadmium, copper, cobalt, and lead. The high result in this case is due to the presence of the cadmium. The polarograms in Figure 4 were obtained with a portion of the 0.2 *N* acid extract of this last preparation. These polarograms fail to show the presence of copper and cobalt and demonstrate that the lead, cadmium, and a small amount of bismuth are carried into the acid extract. The polarographic recovery of zinc in the presence of the above-mentioned metals was satisfactory, as may be seen from the value indicated in the figure and from Table II.

In Table III results obtained by the polarographic and colorimetric methods of analysis of typical materials are compared. The results obtained by the two methods are in satisfactory agreement.

Application of the Methods

These methods have been applied primarily to the analysis of biological material, but they can be applied to other materials as well. They have also been used to determine zinc in electrolytic

Table III. Comparison of Findings by Colorimetric and Polarographic Estimation

Material	Zinc Found: Colorimetric method	Zinc Found: Polarographic method
	Mg. per 24-hour sample	
Feces (1368)	10.5	10.5
Feces (1379)	11.8	11.3
	Mg. per liter	
Urine (680)	0.30	0.31
Urine (1523)	0.20	0.22
Urine (1529)	0.35	0.34
	Mg. per duplicate sample of food consumed in 24-hour period	
Food (1465)	6.4	6.4
Food (1503)	8.25	8.25
	Mg. per 100 grams of tissue (human)	
Cerebellum (790)	0.70	0.65
Lung (792)	0.65	0.70
Pancreas (794)	1.07	1.05
Kidney (798)	1.25	1.15
Blood (1958)	0.45	0.48
Blood (1972)	0.49	0.49
	Mg. per 10 cu. meter of air (in brass foundry)	
Air (10)	16.4	17.0
Air (11)	11.9	11.9

precipitator samples of foundry atmospheres and for the analysis of street dusts. They should be readily adaptable to the analysis of pharmaceuticals and soil samples.

Either method can be used conveniently over a wide range of concentrations, but the great sensitivity of the colorimetric method makes it the one of choice when the quantity of zinc present is known to be very small. Relatively large amounts can be handled more conveniently by polarographic means. The latter method is also more convenient when only occasional samples need be handled and is superior when cadmium is known to be present. Another point in favor of the polarographic method is its superiority whenever it may be desirable to determine lead, bismuth, and cadmium as well as zinc in the material under investigation. In such cases carbamate should not be added in the initial extraction step.

Literature Cited

(1) Bambach, K., and Burkey, R. E., Ind. Eng. Chem., Anal. Ed., 14, 904 (1942).
(2) Biefeld, L. P., and Patrick, T. M., *Ibid.*, 14, 275 (1942).
(3) Cholak, J., *Ibid.*, 7, 287 (1935).
(4) Cholak, J., and Bambach, K., *Ibid.*, 13, 583 (1941).
(5) Cholak, J., and Story, R. V., *J. Optical Soc. Am.*, 31, 730 (1941).
(6) Cowling, H., and Miller, E. J., Ind. Eng. Chem., Anal. Ed., 13, 145 (1941).
(7) Deckert, W., *Z. anal. Chem.*, 100, 386 (1935).
(8) Feicht, F. L., Schrenk, H. H., and Brown, C. E., U. S. Dept. Interior, Bureau of Mines, Rept. Investigation 3639 (1942).
(9) Hibbard, P. L., *Hilgardia*, 13, 1 (1940).
(10) Hibbard, P. L., Ind. Eng. Chem., Anal. Ed., 9, 127 (1937).
(11) Hohn, H., "Chemical Analyses with the Polarograph", Berlin, Julius Springer, 1937.
(12) Holland, E. B., and Ritchie, W. S., *J. Assoc. Official Agr. Chem.*, 22, 333 (1939).
(13) Hubbard, D. M., Ind. Eng. Chem., Anal. Ed., 12, 768 (1940).
(14) Hubbard, D. M., and Scott, E. W., *J. Am. Chem. Soc.*, in press.
(15) O'Connor, R. T., Ind. Eng. Chem., Anal. Ed., 13, 597 (1941).
(16) Reed, J. F., and Cummings, R. W., *Ibid.*, 12, 489 (1940).
(17) Rogers, L. H., and Gall, O. E., *Ibid.*, 9, 42 (1937).
(18) Stout, P. R., Levy, J., and Williams, L. C., *Collection Czechoslov. Chem. Commun.*, 10, 129 (1938).
(19) Suprunovich, I. B., *J. Gen. Chem.* (U. S. S. R.), 8, 839 (1938).
(20) Walkley, A., *Australia Chem. Inst. J. Proc.*, 9, 29 (1942).
(21) Wichmann, H. J., Ind. Eng. Chem., Anal. Ed., 11, 66 (1939).

Determination of Copper in Cast Iron by Direct Microelectrolysis

WILLIAM M. MacNEVIN AND RAYMOND A. BOURNIQUE[1], The Ohio State University, Columbus, Ohio

This investigation of micromethods for the electrolytic determination of copper in nitric acid solutions of cast iron includes: the use of different kinds of apparatus and techniques; the effects of other metals; the effect of nitric, sulfuric, and perchloric acids; the effect of insoluble siliceous and graphitic residues; the molybdenum interference; and the error in sampling. A recommended procedure is given.

THE discovery of important technical properties in copper-iron alloys (*4*) has created a new interest in a rapid and accurate method of analysis for copper. Previously this was not a simple procedure but involved one or more preliminary chemical separations of the copper, followed by electrolysis or other method of determination (*5*, *6*, *9*). The chemical separation, in addition to being slow, was of questionable accuracy unless extreme care was applied.

Various reasons are given (*2*) for the necessity of separating other metals, particularly iron, from copper before it can be satisfactorily determined by electrolysis. (1) If much iron is present with the copper either the voltage used is not great enough to deposit all of the copper or, if it is, some iron usually deposits as well. (2) If nitric acid is present, the following phenomenon frequently occurs (*3*). By the time most of the copper has been deposited, the solution will have acquired a brown color and then the brown color will suddenly disappear, accompanied by a rapid solution of the copper. Further increasing the voltage will not redeposit the copper. This effect has been attributed to the oxidizing effect of ferric ions, nitric acid, ferric nitrate, and oxides of nitrogen. (3) Molybdenum, if present, deposits on the cathode as oxide.

All the variations described above have been observed when conducting, on a macro scale, the electrolysis of copper in nitric acid solutions containing much iron. Preliminary experiments showed that most of the difficulties disappeared when the determination was carried out with microapparatus and enough sample to give 0.3 to 2 mg. of copper.

This suggested the present investigation of micromethods for the electrolytic determination of copper in nitric acid solutions of cast iron. There have been studied: (*a*) the use of different kinds of apparatus and techniques; (*b*) the effects of other metals; (*c*) the effect of nitric, sulfuric, and perchloric acids; (*d*) the effect of insoluble siliceous and graphitic residues; (*e*) the molybdenum interference; and (*f*) the error in sampling.

Samples

A number of solutions containing various amounts of alloying metals were prepared in order to study the individual effect of each metal. After the method was developed it was tested with fifteen samples of cast iron or steel containing varying amounts of copper. These samples are described in Table I and are referred to below according to number.

Table I. Composition of Samples Analyzed

Sample No.	Type	% Cu	% Cr	% Ni	% C	% Si	% Mn	% Mo
1	Cast iron	8.41	4.62	20.20	2.56	1.96	1.22	...
2	Cast iron	8.04	(?)	...	...	..	...	...
3	Cast iron	7.38	2.85	17.32	2.36	1.82	1.08	...
4	Cast iron[a]	6.44	2.18	15.98	2.42	1.60	1.01	...
5	Cast iron	5.96	...	...	3.0	2.0	...	...
6	Cast iron	3.12	...	...	2.8	2.0	...	...
7	Cast iron	3.05	...	...	3.8	1.9	1.46	0.5
8	Cast iron	2.05	...	...	3.4	1.8	1.2	0.2
9	Cast iron	1.91	...	1.0	0.11	..	...	...
10	Cast iron	1.50	...	...	2.90	1.7	1.1	0.5
11	Cast iron[b]	1.44	0.018	0.13	2.86	1.84	0.61	...
12	Cast iron	1.09	1.0	...	...	..	1.0	...
13	Cast iron	0.38	...	...	...	..	...	...
14	Carbon steel[c]	0.267	0.26	0.25	1.03	0.39	0.345	...
15	Chrome-vanadium steel (0.22% V)	0.13	1.03	0.28	0.292	0.21	0.50	...

[a] Bureau of Standards sample 115.
[b] Bureau of Standards sample 5g.
[c] Bureau of Standards sample 35a.

[1] Present address, Westinghouse Electric & Manufacturing Co., Pittsburgh, Penna.

TABLE II. ELECTROLYSIS OF SYNTHETIC SAMPLES

(5-ml. aliquots, H_2SO_4 + trace of HNO_3)

Solution	No. of Aliquots Run	Mean Weight of Cu Observed	Av. Deviation from Mean	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*	*Mg.*
Cu	12	1.391	0.015	1.398	−0.007
Cu	4	1.492	0.015	1.502	−0.010
Cu-Fe	4	1.388	0.006	1.398	−0.010
Cu-Ni	7	1.410	0.007	1.398	+0.012
Cu-Cr	2	1.413	0.000	1.398	+0.015
Cu-Fe-Ni-Cr-Mn	6	1.382	0.017	1.398	−0.016

TABLE III. ELECTROLYSIS IN SULFURIC ACID

(5-ml. aliquots, B. of S. sample 115, 6.44% Cu)

Sample	No. of Aliquots Run	Mean Weight of Cu	Av. Deviation from Mean	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*	*Mg.*
B. of S. cast iron in H_2SO_4	8	1.292	0.005	1.291	+0.001
+ 1 ml. of HNO_3	1	1.291	...	1.291	0.000
+ 2 ml. of HNO_3	1	1.275	...	1.291	−0.016
+ 4 ml. of HNO_3	1	1.288	...	1.291	−0.003
+ 5 ml. of HNO_3	1	1.291	...	1.291	0.000

Apparatus

All the early work was done with the Pregl (*8*) apparatus. A separate study was first made by the authors (*7*) on the precision of the microelectrolytic determination of copper. In doing so the Pregl and Hermance-Clarke (*1*) electrolytic cells were compared. Results with the Pregl cell varied more than could be accounted for and were generally lower than with the Hermance-Clarke apparatus. In the latter cell the solution was drained through a stopcock with continuous addition of wash water, thereby maintaining the circuit until washing was complete. With this apparatus 1 to 2 mg. of copper could be deposited from 7 to 10 ml. of nitric or sulfuric acid solution with an accuracy of ±0.01 mg., a figure that has been used in the present work as the accuracy which may be expected when no interferences occur.

The Hermance-Clarke cell was slightly modified. The surrounding water jacket was omitted and the cell made slightly larger to accommodate 10 to 12 ml. of solution. Otherwise the electrodes, electrode support, and air stirrer were the same.

For smaller percentages of copper, samples 13, 14, and 15, it was necessary to use larger weights of samples. In order to avoid too high a concentration of other metals the solution of the sample was diluted to about 50 ml. and a correspondingly larger cell was used. It was constructed on the principle of the Clarke cell but with a funnel-shaped top to hold the extra solution. In such an apparatus all the solution circulates intimately between the electrodes.

The apparatus was equipped with voltmeter and ammeter with suitable resistances for changing the applied voltage.

The microbalance used was a Sartorius, air-damped type with protected beam. Its sensitivity was 1.35 micrograms per division.

General Procedure

No unusual precautions were taken. Air was bubbled through the solution at a rate of 2 to 3 bubbles per second. Cathodes were cleaned in hot dilute nitric acid, washed with water and alcohol, dried at 105° C. for 5 minutes, and allowed to stand 5 minutes before weighing. Bone-tipped forceps were used in handling the cathodes. Current and voltage were kept as nearly constant as possible during each electrolysis.

Effects of Other Metals

In preliminary work with alloys, results of wide variability and poor accuracy were obtained, but with synthetic solutions containing copper and other metals corresponding in concentration to the samples of cast iron described in Table I, the results (Table II) agreed well with the 0.01-mg. precision obtained with solutions containing only copper. The slightly low results whenever iron was present are not regarded as significant. This comparison was made in solutions containing sulfuric acid with only a trace of nitric, because it is well known that copper can be deposited from such a solution without interference. Deviation from theory could then be attributed to the effect of other metals.

Effects of Nitric, Sulfuric, and Perchloric Acids

It is usually recommended that copper be electrolyzed from a sulfuric acid solution. This is inconvenient because the sample must first be dissolved in an oxidizing acid which is then removed by evaporation. The effect of nitric acid was therefore determined by comparing (Table III) the results using sulfuric acid solutions with those containing various additions of nitric acid. The absence of any trend in the presence of nitric acid is apparent. Without any nitric acid, however, the evolution of hydrogen gas is excessive and produces a spotty deposit.

When nitric acid alone was used the results were equally good. In one set of experiments (Table IV) a copper solution was compared with one containing copper and iron, as a check on the frequent statement in the literature that low results are obtained with nitric acid solutions containing much iron.

In the second set of experiments in nitric acid the copper was determined in a cast iron. The results here (Table V) were slightly high (compare Table III, aliquots in sulfuric acid), which may in part be due to a slight amount of carbon and silica which was not filtered from the individual samples.

Finally, perchloric acid was tried as a solvent for the sample. The rate of solution of the sample was notably slower and evolution of hydrogen was excessive during electrolysis. Some blisters and cavities were observed microscopically on the deposit. When a small addition of nitric acid was made, gas evolution diminished greatly and the deposit was clear. The results obtained (Table VI) have as good precision as those from sulfuric or nitric acid.

TABLE IV. ELECTROLYSIS IN NITRIC ACID

(Synthetic samples, 5-ml. aliquots)

Solution	No. of Aliquots Run	Mean Weight of Cu Observed	Av. Deviation from Mean	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*	*Mg.*
Cu	7	2.024	0.004	2.027	−0.003
Cu-Fe	7	1.997	0.005	2.003	−0.006

TABLE V. ELECTROLYSIS IN NITRIC ACID

(Hermance-Clarke apparatus. Individual samples Bureau of Standards cast iron 115, 6.44% Cu)

Sample Weight	Weight of Cu	Theoretical	Error
Mg.	*Mg.*	*Mg.*	*Mg.*
30.394	1.978	1.957	+0.021
30.946	1.993	1.993	0.000
35.947	2.355	2.315	+0.040
34.073	2.203	2.194	+0.009
29.617	1.934	1.906	+0.028
29.950	1.957	1.929	+0.028
28.499	1.856	1.835	+0.021
37.388	2.407	2.408	−0.001

TABLE VI. ELECTROLYSIS IN PERCHLORIC ACID

(5-ml. aliquots, B. of S. cast iron 115, 6.44% Cu)

Solvent	No. of Aliquots Run	Mean Weight of Cu	Av. Deviation from Mean	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*	*Mg.*
$HClO_4$ + 3-minute boiling	3	1.246	0.003	1.292	−0.046
$HClO_4$ + 15-minute boiling	5	1.290	0.005	1.292	−0.002
$HClO_4$ + 15-minute boiling	2	1.282	0.003	1.292	−0.010

Effect of Insoluble Residues

It was apparent, through most of the work, that results with aliquot samples tended to be lower and in better agreement with the theoretical than with individual samples. In the latter case, any insoluble residue of silica and graphite was not filtered out but was added to the cell, in order to avoid filtration and consequent excessive volumes. When aliquot sampling was employed, the siliceous residue usually settled before samples were taken. It was suspected, however, that some suspended silica or graphite was being coprecipitated with the copper; therefore several experiments were made to check this point. Clear solutions were compared with those in which the suspension was purposely maintained at a maximum (Table VII). It is apparent that high results may be due to this effect. In separate experiments it was found that results were excessively high if the sample was ground unusually fine. In an effort to overcome this effect without the necessity for filtering or allowing the suspensions to settle, additions of gelatin and other organic reagents were made with the purpose of changing any charge on the solid matter and perhaps affecting its attraction by the cathode. Such attempts have been without success so far. The coprecipitation may therefore be largely a mechanical effect.

Interference by Molybdenum

Molybdenum when present was found to deposit on the cathode as the iridescent black sesquioxide, even at voltages as low as 2.2 volts. A solution of phosphoric acid or ammonium bifluoride prevented this. With bifluoride, however, a brownish precipitate formed on the cathode and additional nitric acid was necessary.

TABLE VII. EFFECT OF SILICA AND GRAPHITE

(Sample 1. Best chemical value, 8.41% Cu. Nitric acid solution, 5-ml. aliquots)

Solution	No. of Aliquots	Mean Weight of Cu Observed	Theoretical Weight of Cu	Error
		Mg.	*Mg.*	*Mg.*
1. Clear	6	1.713	1.684	+0.027
1. SiO_2, C suspended	1	1.749	1.684	+0.065
2. Clear (?)	4	1.773	1.726	+0.003
2. SiO_2, C suspended	3	1.837	1.726	+0.078
3. Clear	3	1.689	1.685	+0.004
4. Clear	2	1.714	1.707	+0.007

TABLE VIII. DETERMINATION OF COPPER IN MOLYBDENUM CAST IRON

Sample No.	Mo	Reagent	Theoretical Cu	Observed Cu
	%		%	%
10	0.5	NH_4HF_2	1.50	1.47
		NH_4HF_2		1.50
7	0.5	H_3PO_4	3.05	3.06
		NH_4HF_2		3.01
8	0.2	H_3PO_4	2.05	2.07
		NH_4HF_2		2.04

TABLE IX. ANALYSIS OF SIFTED SAMPLES

Sieve Size	Sample Weight	Weight of Cu		
		Observed	Theoretical	Error
	Mg.	*Mg.*	*Mg.*	*Mg.*
>20-mesh	21.03	1.774	1.769	+0.005
	20.77	1.748	1.747	+0.001
	22.09	1.845	1.858	−0.013
	19.92	1.680	1.676	+0.004
20–40	20.74	1.753	1.744	+0.009
	22.39	1.888	1.882	+0.006
	22.60	1.921	1.900	+0.021
	21.08	1.771	1.773	−0.002
<40	24.34	2.063	2.047	+0.016
	22.80	1.918	1.917	+0.001
	21.40	1.810	1.800	+0.010
	23.58	2.014	1.983	+0.031

TABLE X. DETERMINATION OF COPPER IN INDIVIDUAL SAMPLES OF STEELS AND CAST IRONS

Sample No.	No. of Samples	Approximate Sample Weight	Av. Deviation from Mean	Av. Cu Observed	Theoretical Cu
		Mg.	*Mg.*	%	%
1	12	20–24	0.008	8.44	8.41
2	4	20–23	0.007	7.98	8.04
4	6	20–26	0.016	6.45	6.44
5	4	21–23	0.002	6.07	5.96
9	4	40–43	0.012	1.98	1.91

TABLE XI. DETERMINATION OF COPPER IN ALIQUOT SAMPLES OF STEELS AND CAST IRONS

Sample No.	No. of Aliquots	Weight of Sample per Aliquot	Av. Deviation from Mean Weight of Cu	Av. Cu Observed	Theoretical Cu
		Mg.	*Mg.*	%	%
1	2	20	0.005	8.43	8.41
2	8	20	0.003	7.98	8.04
3	4	20	0.005	7.43	7.38
4	15	20	0.005	6.48	6.44
5	8	20	0.011	6.05	5.96
6	4	25–40	0.004	3.15	3.12
9	2	40	0.004	1.99	1.91
11	4	40–100	0.010	1.52	1.44
12	5	50–80	0.008	1.04	1.09
13	2	80	0.003	0.40	0.38
14	2	200	0.020	0.27	0.27
15	2	200	0.000	0.13	0.13

For a 5-ml. aliquot sample, 1.5 ml. of 85 per cent phosphoric acid or 0.2 gram of bifluoride plus 0.5 ml. of nitric acid was needed in order to prevent the deposition of molybdenum. A voltage of 2.5 volts was sufficient. Three samples of copper-molybdenum cast irons were analyzed with this modification (Table VIII).

Sampling Error

The sampling error is not important if samples as large as 20 to 40 mg. of 20-mesh or finer material are used. Samples were sifted and prepared in three sizes: coarser than 20-mesh, between 20- and 40-mesh, and finer than 40-mesh. Several determinations were made on each of the sifted samples. The results shown in Table IX indicate that individual sampling under these conditions is not a serious cause of error.

Recommended Procedure

Prepare a solution in the necessary solvent, using enough sample so that each 5 ml. will contain 0.3 to 2 mg. of copper. The sample should also contain 0.3 to 0.5 ml. of 70 per cent nitric acid. If the sample contains molybdenum add 0.15 ml. of 85 per cent phosphoric acid. Filter through a sintered-glass micro funnel into the electrolytic cell, rinse, and make up the volume to 9 to 10 ml. Add 3 drops of 95 per cent ethyl alcohol to eliminate spray, adjust the air stream used for stirring to 2 to 3 bubbles per second, and electrolyze at 2.5 to 2.8 volts for 20 minutes. Add 20 to 30 mg. of urea, rinse down the cell walls, and continue electrolysis for another 5 minutes. Slowly drain the cell, adding wash water simultaneously until the current has dropped to zero. Do not allow the voltage to exceed 3.5 volts during this operation. Withdraw the cathode, rinse with alcohol, dry for 5 minutes at 110° C., cool 5 minutes, and weigh.

Table X gives a summary of results obtained in this way using individual samples. Table XI shows similar results using aliquot samples.

Discussion and Summary

The successful electrolytic separation of copper from iron in the presence of nitric acid is attributed to the continuous removal of oxides of nitrogen by the stream of air used for stirring the solution. Nitric acid, which is the most commonly used solvent, therefore need not be removed before carrying out the electroly-

sis. Some nitric acid is, of course, desirable since the nitrate ions prevent excessive evolution of hydrogen gas which would cause a blistered deposit. Sulfuric and perchloric acids do not interfere with the deposition.

The deposits obtained according to the recommended procedure are firm and free from blisters. Qualitative tests have shown them to be free from iron.

The principal interfering metal commonly present is molybdenum, which deposits with the copper as the iridescent black oxide. This is satisfactorily avoided by addition of phosphoric acid or of ammonium bifluoride and some extra nitric acid. Tungsten is ordinarily considered an interfering element, but since it is removed as the insoluble oxide after the sample has been treated with nitric acid, it does not affect the electrolysis. Carbon and silica have been found embedded in the deposit and the deposits were notably heavy in the cases where these insoluble materials were left suspended in the solution of the sample. Simple filtration through a fritted glass microfilter, as part of the process of transferring the sample to the electrolytic cell, has eliminated this difficulty.

The precision of the separation of 0.3 to 2 mg. of copper from aliquot samples of solution of copper containing steels and cast irons is of the order ±0.01 mg. This value is only slightly larger when individual samples are analyzed, showing that sampling is not a serious problem.

The time required for depositing 0.3 to 2 mg. of copper under the conditions described in this paper is not in proportion to the time required for macro amounts of copper, but is relatively greater. Although it has not been possible to shorten the time to less than 25 to 30 minutes, the procedure is definitely more rapid than those employing chemical separations.

Literature Cited

(1) Clarke, B. L., and Hermance, H. W., *Bell System Tech. Publ.*, Chem. B, No. 658 (1931); *J. Am. Chem. Soc.*, 54, 877 (1932).
(2) Classen, A., "Quantitative Analysis by Electrolysis", 7th German ed., pp. 156, 308, 347, 350, Berlin, Julius Springer, 1927.
(3) Fairlie, A. M., and Bone, A. J., *Electrochem. Metallurgical Industry*, 6, 58 (1908).
(4) Gregg, J. L., and Daniloff, B. N., "Alloys of Iron and Copper", 1st ed., New York, McGraw-Hill Book Co., 1934.
(5) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis", p. 197, New York, John Wiley & Sons, 1929.
(6) Lundell, G. E. F., Hoffman, J. I., and Bright, H. A., "Chemical Analysis of Iron and Steel", New York, John Wiley & Sons, 1931.
(7) MacNevin, W. M., and Bournique, R. A., Ind. Eng. Chem., Anal. Ed., 12, 431 (1940).
(8) Pregl, F., "Quantitative organische Mikroanalyse", 3rd ed., p. 185, Berlin, Julius Springer, 1930.
(9) U. S. Steel Corp., "Sampling and Analysis of Carbon and Alloy Steels" (Methods of Chemists of Subsidiary Companies of U. S. Steel Corp., Revised to 1937), p. 116, New York, Reinhold Publishing Corp., 1938.

Microdetermination of Glycolic and Oxalic Acids

VINCENT P. CALKINS, Biochemical Laboratory, State University of Iowa, Iowa City, Iowa.

A quantitative colorimetric micromethod for glycolic acid has been devised from a sensitive qualitative spot test based on the color produced with 2,7-dihydroxynaphthalene in acid solution. The same reaction may be employed for the quantitative microdetermination of oxalic and glyoxylic acids after reduction with powdered magnesium. Glycolic acid appears to be an intermediate product in the oxidation of ascorbic to oxalic acid.

FOR use in a study of the stabilizing action of ascorbic acid in certain fats (*2*), a micromethod for the quantitive estimation of oxalic acid was sought. Oxalic acid is a postulated oxidation product of ascorbic acid (*1*) and might be the substance ultimately responsible for its antioxygenic action. On controlled reduction with powdered magnesium, oxalic acid yields glycolic acid (*3*).

This paper describes the application of a color test for glycolic acid to the microestimation of glycolic and oxalic acids. By a suitable modification glyoxylic acid can also be determined, since it is likewise reduced to glycolic acid with powdered magnesium (*3*). When glyoxylic acid is present with oxalic acid in the same solution, the latter can be removed by precipitation from neutral solution by gypsum water.

When a solution of glycolic acid reacts with a solution of 2,7-dihydroxynaphthalene in concentrated sulfuric acid, a violet-red color is produced on heating; according to Eegriwe (*3*), who first described this reaction, the color probably depends on the condensation of formaldehyde, split off from the glycolic acid, with dihydroxynaphthalene to form tetrahydroxydinaphthylmethane; this colorless product is then gradually oxidized to a deep red dyestuff. None of the more common organic acids interfered with the test, including formic, acetic, oxalic, succinic, tartaric, citric, benzoic, and salicylic.

Color measurement is made on a calibration curve prepared from known solutions of glycolic acid and read in a Coleman spectrophotometer at 530 mμ or a Klett-Summerson photoelectric colorimeter with filter No. 52. Figure 1 shows the spectral transmittance curve of several concentrations of glycolic

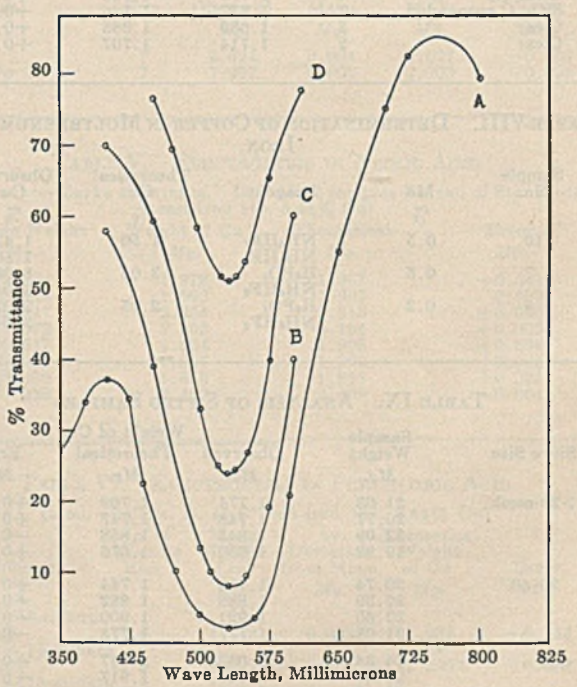


Figure 1. Spectral Transmission Curve

Micrograms of glycolic acid: *A*, 40; *B*, 20; *C*, 10; *D*, 5

acid. The calibration curves of glycolic acid (logarithmic) and of oxalic acid were both straight lines within the ranges used.

Determination of Glycolic Acid

The solution to be analyzed should be 2 *N* with sulfuric acid and should contain not over 100 micrograms of glycolic acid per ml. Into a 15-ml. conical glass-stoppered centrifuge tube, 0.2 ml. of this solution is introduced with a serological pipet, and 2 ml. of 0.01 per cent solution of 2,7-dihydroxynaphthalene in concentrated sulfuric acid are added from a microburet, the tube being kept in a cold-water bath during the addition of the reagent. The mixture is made homogeneous by rotating the tube between the hands, and the tube is then placed in a boiling water bath for 20 minutes, removed, and allowed to cool to room temperature. After cooling, the solution is diluted with 4 ml. of 2 *N* sulfuric acid and shaken, lightly at first, until the heat of reaction has subsided. The solution is made homogeneous by vigorous shaking and the color is then read.

The method is very flexible; after the color has been formed, practically any dilution volume can be used if it is preferable to reduce the sensitivity of the method so that higher concentrations of glycolic acid can be determined. Even the volume of test solution can be changed within narrow limits.

Determination of Oxalic Acid

This differs from the preceding only in that to the original sample as measured out (0.2 ml. of a 2 *N* sulfuric acid solution containing not more than 200 micrograms of oxalic acid) 5 mg. of powdered magnesium are added; after rotating between the palms of the hands, the tube is allowed to stand for an hour. Then with the tube in cold water, 2 ml. of the dihydroxynaphthalene solution are added, and after being made homogeneous, the solution is heated on the boiling water bath for 20 minutes, removed, and allowed to cool to room temperature. It is then diluted with 4 ml. of 2 *N* sulfuric acid, mixed, and the color is measured.

The calibration curve shows that Beer's law is obeyed from 0 to 200 micrograms of oxalic acid, and even with this range the method is sensitive at low concentrations (0 to 20 micrograms).

Literature Cited

(1) Borsook, Henry, Davenport, H. W., Jeffreys, C. E. P., and Warner, R. C., *J. Biol. Chem.*, 117, 237 (1937).
(2) Calkins, V. P., and Mattill, H. A., *J. Am. Chem. Soc.*, in press.
(3) Eegriwe, E., *Z. anal. Chem.*, 89, 12 (1932).

Micromethod for Estimation of Sulfonamides

Semipermanent Color Standards

S. W. LEE AND N. B. HANNAY

Wallace Laboratories, Inc., New Brunswick, N. J.

AS A supplement to the method for sulfonamide determination previously reported (*1*), a simple procedure has been worked out, using color standards prepared from easily available, water-soluble dyes. It is felt that this modification will be of use especially in areas remote from laboratory facilities. Acceptable accuracy has been obtained to blood levels of 15 mg. per cent (for sulfathiazole). By averaging the results of several observers, it was found that the error (for sulfathiazole) was ±0.5 mg. per cent to levels of about 6 mg. per cent, and ±1 mg. per cent at higher levels to 15 mg. per cent.

The advantages of the parent micromethod have been retained, though the procedure has been simplified. The number of manipulations and the required apparatus are minimal.

The volume of blood needed (0.3 ml.) may be taken from the finger tip, if the hand of the patient has been vigorously washed to remove traces of the sulfa drug. Flat-bottomed 10-ml. vials (18 × 46 mm.), calibrated for the purpose of the method, are to be preferred to centrifuge or test tubes.

The standards of known sulfonamide concentration may be made from any of the sulfonamides, and the values for the others obtained by using molecular weight corrections. The values obtained for sulfathiazole may be multiplied by 0.68 to convert to values for sulfanilamide, while those for sulfapyridine are the same as for sulfathiazole.

PROCEDURE. The same reagents and procedure are used as in the parent method (*1*), but only the blood and precipitating solution volumes need be measured with accuracy. The nitrite and dye solution (2 drops or 0.1 ml.) may be added from a dropper. Comparisons of the tubes from the determinations with the standards are made visually, always using vials of uniform shape. It was noted that the eye best measures the intensities of transmitted light if the absorbing substances be held 1 to 3 cm. from the eye. One should look through the solution.

COLOR STANDARDS FOR COMPARISONS. Semipermanent fuchsin–methyl violet color standards were prepared as follows:

Acid fuchsin (0.100 gram) was dissolved in 250 ml. of distilled water, and this was diluted 1 to 8 with water, giving a solution containing 0.00005 gram in 1 ml. Methyl violet (0.100 gram) was dissolved in 250 ml. of water, and 2 ml. of this solution were made up to 250 with water (0.0000032 gram in 1 ml.). These solutions were mixed and diluted as shown in Table I to give color intensities corresponding to the indicated amounts of sulfathiazole.

TABLE I. PREPARATION OF COLOR STANDARDS

Standard	Fuchsin Solution	Methyl Violet Solution	Water
Mg. %	*Ml.*	*Ml.*	*Ml.*
2	0.9	9.1	36.0
3	0.9	9.1	25.3
4	0.9	9.1	19.0
5	0.9	9.1	14.7
6	0.9	9.1	11.2
7	0.9	9.1	8.8
8	0.9	9.1	7.0
9	0.9	9.1	5.4
10	0.9	9.1	3.9
11	0.9	9.1	2.9
12	0.9	9.1	2.1

Several combinations of red and violet water-soluble dyes gave shades almost identical to that of the azo color formed in the reaction. Colors were matched with the eye. The dye combination is, of course, bleached by sunlight, and those in use lasted for about a month. Standards should be kept in the dark when not in use. Stronger solutions of the dyes keep longer than the weak ones, and may be kept for subsequent diluting.

Literature Cited

(1) Lee, S. W., Hannay, N. B., and Hand, W. C., IND. ENG. CHEM., ANAL. ED., 15, 403 (1943).

Microdetermination of the Formyl Group

JOSEPH F. ALICINO, Squibb Institute for Medical Research, New Brunswick, N. J.

WHILE the microdetermination of the acetyl group is now widely used, little attention has been paid to analysis of the formyl radical. Usually identification of the formyl derivative by means of carbon and hydrogen analysis is sufficient, but in certain critical cases an analysis of another constituent may be desirable.

The acetyl determination should be valid for all volatile acyl groups, but since no reference to the application of this method to the formyl group was found in the literature, this determination was studied.

Because it was felt that the reducing properties of formic acid might serve to distinguish it from the other volatile acids, the question of whether formic acid and bromine react quantitatively in 0.01 *N* solutions was investigated. It was found that the formyl group can be determined under the same conditions as the acetyl group. The determination can be made specific by treating the distilled formic acid with an excess of bromine.

Formic acid solution, 0.01 *N*, was prepared and standardized by means of excess potassium iodate and potassium iodide, and 0.01 *N* bromine solution was prepared and standardized by titration of iodine from potassium iodide. This solution must be stored in a refrigerator and its factor checked frequently. An excess of the bromine solution was added to a measured amount of formic acid in a stoppered flask and shaken thoroughly, and after 5 minutes the excess bromine was determined (Table I).

TABLE I. DETERMINATION OF FORMIC ACID

0.0102 *N* Formic Acid	0.00982 *N* Bromine Solution	Calculated Excess	Observed Excess	Difference
Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*
10.00	15.00	4.61	4.58	+0.03
10.00	15.00	4.61	4.64	−0.03
10.00	15.00	4.61	4.57	−0.04
5.00	10.00	4.81	4.82	+0.01
5.00	10.00	4.81	4.78	−0.03
5.00	10.00	4.81	4.77	−0.04

TABLE II. DETERMINATION OF FORMYL

Substance	Formyl Found as Formic Acid	Formyl Found as Reducing Agent	Formyl Calculated
	%	%	%
Triformyl cholic acid	17.19 17.30	17.33	17.68
Diformyl desoxycholic acid	12.56 12.66	12.43	12.94
Cholesteryl formate	7.01 6.88	7.25	7.00
S-Benzyl-*N*-formyl-*dl*-cysteine	12.38	...	12.15
S-Benzyl-*N*-formyl-*d*-cysteine	12.20 12.05	...	12.15
S-Benzyl-*N*-formyl-*l*-cysteine	12.15 12.20	...	12.15
Methyl formaniline	21.36 21.32	...	21.48

Determinations were run according to the Elek-Harte method for acetyl (*2*) on several formyl derivatives whose purity was checked by melting point and carbon and hydrogen analysis (Table II). In a few instances where there was available material, the modified method using the liberated formic acid as a reducing agent was followed. It was observed and substantiated by the senior author (*1*) that correction for the amount of sulfur dioxide liberated from the saponifying agent is seldom necessary, if the procedure is carefully controlled. Therefore, freshly distilled water can be used in the receiver where there is a question of the nature of the acid group. In this case, the distillate is quantitatively transferred to a stoppered flask, the properly prepared bromine solution is quickly added in excess, and the excess is titrated as described. If both formyl and acetyl may be present, a total acid titration should first be performed, preferably on a separate sample or on an aliquot of the distillate.

Acknowledgments

The author wishes to express his thanks to Oskar Wintersteiner for his advice and interest in this work.

The cysteine derivatives were kindly furnished by J. A. Stekol.

Literature Cited

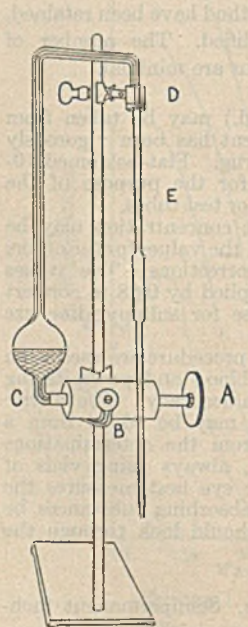
(1) Elek, A., private communication.
(2) Elek, A., and Harte, R. A., IND. ENG. CHEM., ANAL. ED., 8, 267 (1936).

Pipetting Apparatus

W. G. SCHNEIDER, Mallinckrodt Chemistry Laboratory, Harvard University, Cambridge, Mass.

DURING a recent research, it was necessary to withdraw accurately aliquots of 2 and 3 cc., respectively, from a two-phase liquid system containing 3 cc. of aqueous solution and 4 cc. of a chloroform solution. The solute, whose concentration in each of the two phases was to be determined, was highly toxic. In addition to the hazards involved in carrying out the operation by the usual pipetting technique, it was difficult to obtain a clean separation of the layers. The apparatus described below, while originally designed to fill these needs, has, because of its simplicity and ease of operation, been found extremely useful for all pipetting operations, and particularly for pipetting strong acids and bases, toxic liquids, and volatile solvents. The apparatus provides smooth, accurate control, and the speed of pipetting is comparable to that by the usual technique.

The brass cylinder, *B* (2.5 cm., 1 inch, in inside diameter), receives a threaded brass plug, *A* (threaded surface 2.5 cm., 1 inch, long); 16 threads per inch have been found convenient. The capacity of the cylinder assembly is 25 cc. Pyrex capillary tubing (2-mm.), joined to the opposite end of the cylinder by means of a suitable cement (Shawinagan resin, Picein, or DeKhotinsky cement), is sealed to a glass bulb, *C*, of 25-cc. capacity, and the latter joined to a further length of capillary tubing as shown in the diagram. *D* is a No. 1 one-holed stopper which receives the pipet. About 30 cc. of glycerol are introduced into the apparatus; air trapped in *B* can be removed by holding the apparatus on its right side and turning up plunger *A*. The glycerol besides lubricating the threaded surface acts as a convenient air block, making the apparatus airtight.



To operate the apparatus, the pipet to be used is moistened and inserted into stopper *D*. By turning *A* counterclockwise, the liquid to be pipetted is lifted slightly above the pipet mark, *E*. With the tip of the pipet resting against the side of the vessel containing the liquid, *A* is turned slowly clockwise until the liquid level reaches mark *E*. Delivery is then effected by clockwise rotation of *A*. Any volume of liquid from 25 cc. to less than 0.01 cc. can be accurately measured.

A small-diameter, knurled extension attached to disk *A* may be found convenient for major adjustments, and will considerably reduce the time required for operation.

NOTES ON ANALYTICAL PROCEDURES

Substitute for Benzene in the Determination of Acid Numbers

P. L. GORDON, M. A. GILDON, AND F. L. RUBIN
Capitol Paint & Varnish Works, Inc., Brooklyn, N. Y.

The aromatic petroleum solvent Solvesso No. 1 is submitted as a substitute for benzene in acid number determinations on oils, resins, and related products. The physical properties of the two solvents are tabulated and a comparison is presented of their action as solvents in the acid number titration of several paint vehicle ingredients.

IN THE determination of the acid number of oils, resins, and varnishes as outlined in the A. S. T. M. standards on paint, varnish, lacquer, and related products, the customary solvent is a mixture of C. P. benzene and 95 per cent ethyl alcohol (*1*). Essentially, the procedure involves the titration of free acid with standardized alcoholic potassium hydroxide using phenolphthalein as indicator, and the results are reported as milligrams of potassium hydroxide per gram of oil, resin, or varnish.

The authors' laboratory has frequent occasion to run acid number tests, and had standardized on a mixture of equal parts of C. P. benzene and denatured anhydrous ethyl alcohol. Owing to the exigencies of war, it was found advisable to secure a substitute for the benzene.

A study of the literature disclosed that a number of other solvents and solvent combinations had been investigated. Methyl, isopropyl, and amyl alcohols were substituted for ethyl alcohol (*2*, *4*, *6*); however, these substitutions did not eliminate the necessity of using benzene to ensure a suitable range of solubility. Ether and chloroform (*3*, *4*) were suggested in combination with ethyl alcohol, but the investigation covered a narrow range of materials and did not indicate their usefulness in the case of synthetic resins and natural gums.

Because of its availability and physical properties, the solvent selected for examination was the aromatic petroleum product, Solvesso No. 1, supplied by the Colonial Beacon Oil Co. (*5*). A comparison of its physical and solvency characteristics with those of C. P. benzene is presented in Table I. Common paint vehicle ingredients were checked for acid number in the following solvent mixtures (results are given in Table II):

A. 50% C. P. benzene
   50% denatured anhydrous alcohol
B. 50% Solvesso No. 1
   50% denatured anhydrous alcohol
C. 66.6% Solvesso No. 1
   33.3% denatured anhydrous alcohol

The mixture of 2 parts of Solvesso No. 1 to 1 part of denatured anhydrous ethyl alcohol proved to be an effective solvent for the materials commonly checked for acid number. In the case of heavy bodied linseed oil, the standard benzene-alcohol mixture turned extremely cloudy during titration, while mixture C maintained a clear solution throughout the titration, permitting a more rapid introduction of potassium hydroxide solution and yielding a sharper end point. In all determinations, no interference could be detected with either the titration or the end point, and it is concluded that the Solvesso No. 1–alcohol combination is a satisfactory substitute for the benzene-alcohol standard.

TABLE I. PHYSICAL PROPERTIES OF C. P. BENZENE AND SOLVESSO NO. 1

	C. P. Benzene	Solvesso No. 1
Specific gravity	0.884	0.815
Boiling range	80–80.5° C.	93.3–135° C. 90% off at 118.8° C.
Kauri-butanol value	120	72.5
Mixed aniline point	7.3° C.	26.7° C.

TABLE II. ACID NUMBER DETERMINATION USING SOLVENT MIXTURES

Sample	Solvent Mixture A	B	C
Oils			
Raw linseed oil	3.8	3.8	3.8
Bodied linseed oil, high acid	19.1[a]	19.2[a]	19.2
Resins			
Gum rosin	168	170	169
Limed wood rosin	81.0	82.3	81.1
Maleic resin	14.5	14.5	14.5
Alkyd resin	14.7	14.6	14.7
Ester gum	7.9	8.0	8.0
Run kauri	108	107	107
Run East India	16.5	16.7	16.7
Manila	123[a]	123[a]	124[a]
Coumarone-indene resin	Neutral	Neutral	Neutral
Varnishes			
Limed wood rosin Linseed oil, 50 gallon oil length	17.0[a]	17.0[a]	16.9
Ester gum–linseed oil, 20 gallon oil length	2.9	2.8	2.8
Phenol formaldehyde–tung oil–linseed oil, 25 gallon oil length	9.7	9.6	9.7

[a] These samples yielded cloudy solutions.

Literature Cited

(1) Am. Soc. Testing Materials, Standards on Paint, Varnish, Lacquer, and Related Products, pp. 137, 181 (Jan., 1941).
(2) Ellis, C., "Chemistry of Petroleum Derivatives", p. 365, New York, Chemical Catalog Co., 1934.
(3) Gardner, H. A., "Physical and Chemical Examination of Paints, Varnishes, and Colors", 9th ed., pp. 286, 329, Institute of Paint and Varnish Research, 1939.
(4) Lewkowitsch, J., "Chemical Technology and Analysis of Oils, Fats, and Waxes", 6th ed., Vol. 1, p. 446, London, Macmillan Co., 1921.
(5) Mantell, C. L., "Natural Resin Handbook of American Gum Importers Association", p. 68, 1939.
(6) Mattiello, J. J., "Protective and Decorative Coatings", Vol. 1, p. 202, New York, John Wiley & Sons, 1941.

Sintered Pyrex and Soft Glass Sections in Tubes and Crucibles

G. N. QUAM AND JOHANNA HENN[1]
Long Island University, Brooklyn, N. Y.

SINCE the report on porcelain filter mats (*10*) and Pyrex filter mats (*11*), many contributions have appeared on the construction and use of Pyrex sintered ware. In general the procedures suggested have involved sealing in place a prepared and shaped disk of sintered Pyrex, porcelain, or Alundum under controlled heating in a furnace (*1*, *2*, *3*, *5*, *7*, *8*). In others the sintered-glass sections are prepared in tubes in open flames (*4*, *6*, *9*).

The reason for so many contributions is no doubt the efficiency and ease of use of sintered-glass or porcelain ware in gas dispersers, gas wash bottles, mercury filters and valves, stills, absorbers, extraction thimbles, filters for fine precipitates, and aerators.

The advantages of construction of a sintered mat directly in the tube or crucible in one operation have been demonstrated by the senior author. The graded glass may be packed in any part of the tube and sintered in place. The authors have not observed any reference to their method and therefore add this report on procedure and technique for the benefit of those who wish to construct their own apparatus for preliminary or exploratory observations.

Preparation

The glass was ground in a steel mortar and graded by means of a set of sieves, 10- to 200-mesh. The graded glass was treated with 6 *N* hydrochloric acid, washed, and dried. For the first heating the mat was prepared by placing an asbestos disk at the lower level and holding it in place by fine asbestos fiber packed from below. The graded glass was poured on to the desired thickness. For the construction of a filter mat this first layer consisted of a mixture of 40 per cent of 40-mesh, 35 per cent of 80-mesh, and 25 per cent of 120-mesh. The mat was wetted with a few drops of *N* sodium hydroxide (sodium silicate solution, specific gravity 1.036, also served as a suitable flux). Without the flux many mats failed to fuse uniformly to the wall of the tube or crucible. After this sturdy mat had been sintered in place, the process of controlling porosity followed. The finer grades (130- to 200-mesh) were kept suspended in the diluted fluxing solution and pulled into the interstices by suction. thus building up an effective filter bed. The smaller particles were then sintered.

Control of Sintering Process

The approximate temperature or cone number required for sintering was determined with cones made from the glass by drawing a short section of tubing to a point. These glass "cones" were placed in the furnace along with a selected series of pyrometric cones, or a thermocouple. The approximate temperature at which the glass would sinter was thus determined in terms of cone number or thermocouple range. For the work reported here cone No. 0.020 was found suitable for the soft glass and cone No. 0.015 for the Pyrex, while 625° C. (thermocouple) was suitable for the soft glass and 745° C. for Pyrex.

The glassware was made ready for the furnace by packing the glass tubes or crucibles in fire-clay crucibles with fine asbestos fiber. Distortion was reduced to a minimum by maintaining the tubes in a vertical position by loose uniform packing. Sturdy sintered mats were obtained with soft glass when the temperature was allowed to rise slowly to 625° C. and held there for 15 minutes and with Pyrex when the temperature was held at 745° C. for 25 minutes.

Cleaning of Sintered Mats

The sintered mats were washed with water, dried, and weighed (weight A, Table I). Any soluble substances remaining from the sodium hydroxide or sodium silicate treatment were extracted with 6 *N* hydrochloric acid in the following manner: The mat was placed in the acid for 24 hours, then washed, dried, and weighed (weight B, Table I); the process was repeated to determine further loss (weight C).

Pressing the mats with a stirring rod indicated no apparent loss in sturdiness from the acid extraction. Walls of split mats examined under a low-power microscope, equipped with an illuminator, showed that the porosity was uniform and that the structure was continuous.

The method of preparing built-in sintered mats has been successful for a wide variety of grades of powdered glass. Sections limited by the size of muffle of the furnace can be joined to larger pieces of glass. The coarser grades can be heated to slightly higher temperature with resulting increase in sturdiness. The authors suggest this method only as a device for preparing sintered-glass mats during exploratory experimentation in regular laboratory procedure or research. The amateur glass worker should rely on experts for the construction of permanent glass equipment of special design.

TABLE I. WEIGHT LOSSES FROM HYDROCHLORIC ACID TREATMENT

Thickness of Mat *Cm.*	Weight A *Grams*	Weight B *Grams*	Loss *Mg.*	Weight C *Grams*	Loss *Mg.*
Soft glass					
1.0	8.5895	8.5852	4.3	8.5852	0.0
0.9	6.9514	6.9482	3.2	6.9483	−0.1
1.0	8.3727	8.3692	3.5	8.3692	0.0
1.1	7.6223	7.6193	3.0	7.6195	−0.2
Pyrex					
0.7	6.3704	6.3690	1.4	6.3690	0.0
0.5	5.8145	5.8131	1.4	5.8130	0.1
0.7	4.8661	4.8647	1.4	4.8648	−0.1
1.0	8.1299	8.1278	2.1	8.1277	0.1

Literature Cited

(1) Bruce, W. F., and Bent, H. E., *J. Am. Chem. Soc.*, 53, 990 (1931).
(2) Degering, E. F., IND. ENG. CHEM., 24, 181 (1932).
(3) Holmes, F. E., *J. Chem. Education*, 18, 311 (1941).
(4) Kirk, P. L., IND. ENG. CHEM., ANAL. ED., 7, 135 (1935).
(5) Kirk, P. L., Craig, Roderick, and Rosenfels, R. S., *Ibid.*, 6, 154 (1934).
(6) Laitinen, H. A., *Ibid.*, 13, 393 (1941).
(7) Prausnitz, Paul H., IND. ENG. CHEM., 16, 370 (1924).
(8) Roswell, C. A., IND. ENG. CHEM., ANAL. ED., 12, 350 (1940).
(9) Shatenshtein, A. E., *J. Chem. Ind.* (*Moscow*), 6, 1800 (1929).
(10) Sweeney, O. R., and Quam, G. N., *J. Am. Chem. Soc.*, 46, 958 (1924).
(11) Wilkinson, J. A., and Quam, G. N., private communications, August, 1924.

[1] Present address, Iowa State College, Ames, Iowa.

The Use of Filter Paper Disks with Gooch and Porous-Type Crucibles in Routine Filtrations

J. M. FULTZ, Frochling & Robertson, Inc., Richmond, Va.

THE acute shortage of Gooch-grade asbestos fiber has made it necessary for all laboratories to devise means for conserving their diminishing supply, or to turn to other less satisfactory means of quantitative filtration. The following procedure has proved of value as a time-saver and as a means of conserving asbestos fiber, especially when routine filtrations of precipitates such as barium sulfate or magnesium pyrophosphate are in order.

Where a limited supply of asbestos is still available, a Gooch crucible is made up in the ordinary manner, ignited, cooled, and weighed. Just before filtering, a filter paper disk (24 mm. in diameter for 30-ml. crucibles) is placed on the mat in the crucible and washed once with distilled water to secure it as firmly as possible against the mat. [Carl Schleicher & Schüell Co.'s No. 895-E ashless filter mats have proved very satisfactory for the use described.] The filtration is then carried out in the usual manner, and the crucible is ignited, cooled, and weighed. Immediately after weighing, the precipitate is removed mechanically, and the crucible is reweighed and thus is immediately ready for reuse.

To remove the precipitate, the crucible is inverted and gently tapped. Most of the nonadhering precipitate will drop out, since the filter paper disk prevents the adherence of the precipitate to the filter mat, whether it be asbestos, fritted glass, or porcelain. Should any precipitate adhere, after it is gently loosened with the aid of a spatula, it can be brushed out with a camel's-hair balance brush.

The procedure with modifications has been applied with equal satisfaction in the use of the porous-type filtering crucible and the time saved in cleaning has been considerable. In using this type of crucible the filter paper disk (24 mm. in diameter for 30-ml. crucibles) is inserted just before filtering and, as with the Gooch crucible, the precipitate is removed mechanically just after weighing. The crucible is then reweighed and ready for reuse.

The success of the use of the filter paper disks seems to be brought about by the following action during ignition within the crucible. The paper prevents adherence of the ignited precipitate to the asbestos mat or to the filtering medium of the porous-type crucible by the expulsion of the volatile organic matter during the combustion. It also prevents the clogging of the asbestos mat or the filtering medium by the material being filtered.

The procedure can be used for any ignited precipitate, whether it be capable of removal by solution or not. The removal of most filtered solids by solution is time-consuming, and doubtful as to completeness. The method described provides a crucible, weighed and ready for another filtration in very short order, with no impairment to the filter mat by occlusions.

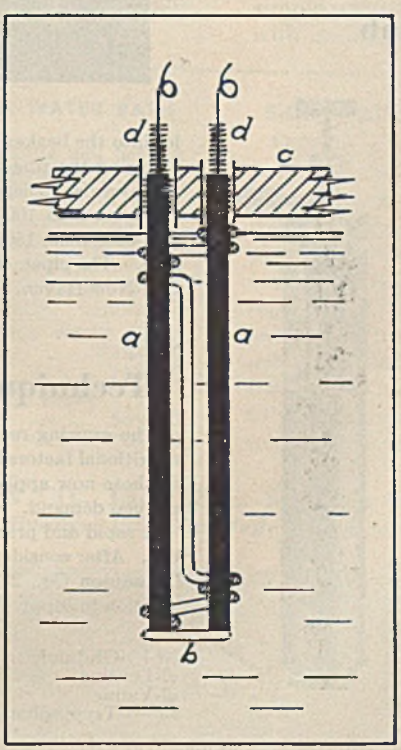
Electric Heating Unit

GABOR B. LEVY, Schenley Research Institute, Lawrenceburg, Ind.

IT IS frequently necessary to provide apparatus with a bath containing boiling water in order to maintain a temperature of approximately 100° C. In order to heat up and maintain the liquid at the boiling point, an electric heater can be used to advantage.

Since it is rather difficult, at present, to procure suitable heaters, the construction of a heating unit in the laboratory may often become imperative. A simple electrical heater can be assembled from readily available materials by using the liquid of the bath as resistance. In such heaters, the rate of boiling is controlled by the quantity of electrolyte present, the dimensions and distance of the electrodes, and the voltage applied. If available, a variable resistance or variable transformer may therefore be included in the circuit to facilitate control of the rate of heating. A heater can be operated satisfactorily, however, without the use of either of these devices. In this case, the exact concentration of the electrolyte for the desired rate of heating has to be determined empirically.

On this basis a heating unit was assembled as follows:



Two spectroscopic carbon rods, *a*, of 5-mm. diameter and 150-mm. length are immersed in the bath and rigidly fixed at a distance of approximately 10 mm. by means of a glass holder, *b*, as shown in the figure.

The holder is made by bending a glass rod of 3-mm. diameter into proper shape. The carbon rods are passed through glass tubes which are inserted into the cover, *c*, of the water bath. The leads of the 110-volt alternating current line are forced to the carbon rods by means of spiral springs, *d*.

Approximately 0.01 *N* sodium chloride solution is used as the electrolyte solution.

The water bath itself is a closed system, provided with a reflux condenser.

In an apparatus for the analysis of butadiene (*1*) a heating unit of the above description has been substituted and in use for several months. The main advantages are its small space requirement and the fact that it can be assembled in little time and without the use of critical materials.

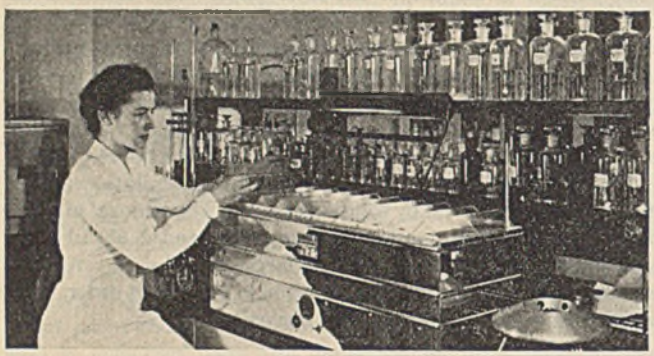
Literature Cited

(1) Tropsch, H., and Mattox, W. J., IND. ENG. CHEM., ANAL. ED., 6, 104 (1934).

NEW LABORATORY EQUIPMENT

Plastic Cover

Plastics has replaced another strategic metal, not only for the duration but also for the postwar period. A gable-type cover, devised for the U. S. Army Medical Corps serological bath, has the advantage over the former copper cover in being transparent as well as resistant to corrosion, and having a low coefficient of heat transfer which makes it an excellent thermal insulator to assist in maintaining constant temperature inside the water bath.



When these covers were put to use, moisture condensed on the inside, reached the saturation point, then abruptly ran down to the lower edges of the covers and dropped off into the water bath, instead of onto the test tubes, as with metal covers.

The equipment is being used by the Army for its Wassermann and Kahn tests with blood samples incubated at constant temperatures of 37° and 56° C. Molded of polystyrene, the cover was developed by the Precision Scientific Co., 1750 North Springfield Ave., Chicago 47, Ill.

Preliminary Test Bomb

A test bomb has been developed by the American Instrument Co., Silver Spring, Md., for making preliminary tests of chemical reactions at high pressures.

As normally supplied, the pressure seal is made by a copper gasket, totally enclosed for safety, between the head and the mouth of the bomb. Screwed into a standard Aminco superpressure opening for 0.25-inch outside diameter tubing in the head is a needle valve with side outlet for the same type of construction.

No opening is normally supplied in the bottom of the bomb. Where it is desired to maintain flow within the chamber, the bomb can be supplied with a standard connection, with plug to seal the bottom opening when not in use.

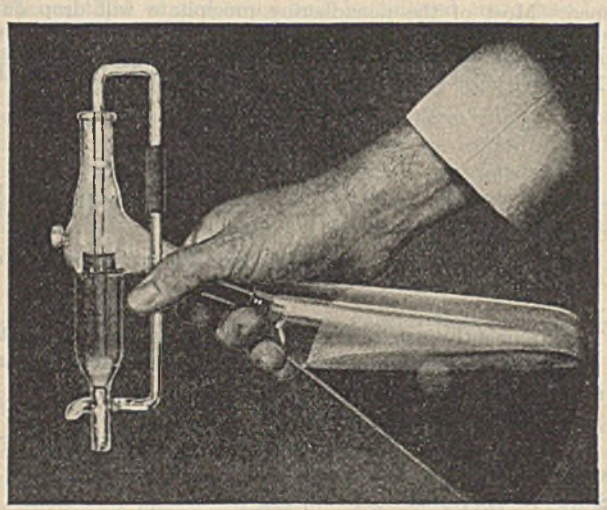
Standard construction is of high-strength steel. Each bomb is tested individually to 15,000 pounds per square inch and is intended for safe operation at a maximum internal pressure of 10,000 pounds per square inch. Calculated bursting pressure is over 40,000 pounds per square inch. The valve in the head permits charging at pressures as high as 5000 pounds per square inch and will seal the contents of the bomb up to the full working pressure of 10,000 pounds per square inch.

Water Testing Equipment

A test set for boiler water control analyses is announced by W. H. and L. D. Betz, Gillingham and Worth Sts., Frankford, Philadelphia, Penna. The set includes all the necessary apparatus and chemicals for the determination of hardness, alkalinity, chloride, sulfite, and phosphate. A special cabinet designed for use on table or wall holds apparatus and chemicals in a secure position and ready for instant use. A portion of the opened cabinet door forms a convenient acid-resistant laboratory work table and a fluorescent light provides correct illumination for the tests.

Automatic Pipet

In the automatic pipet illustrated, the measuring chamber is filled (slightly to overflowing) by tilting the flask to pouring position. When the flask is returned to vertical, the surplus drains back, leaving the chamber with an exactly predetermined volume. Its content is



let into the beaker by raising the plunger-lever with the thumb or by engaging the ring-hook with the far rim of the beaker and pressing downwards. Dispenser assemblies may be had for pipetted quantities from 5 to 100 cc., each dispenser operating with flasks holding 250, 500, 1000, 1500, or 2000 cc. Special combinations are also available. The pipet, catalog No. 850, is made by the Macalaster Bicknell Co., New Haven, Conn., or Cambridge, Mass

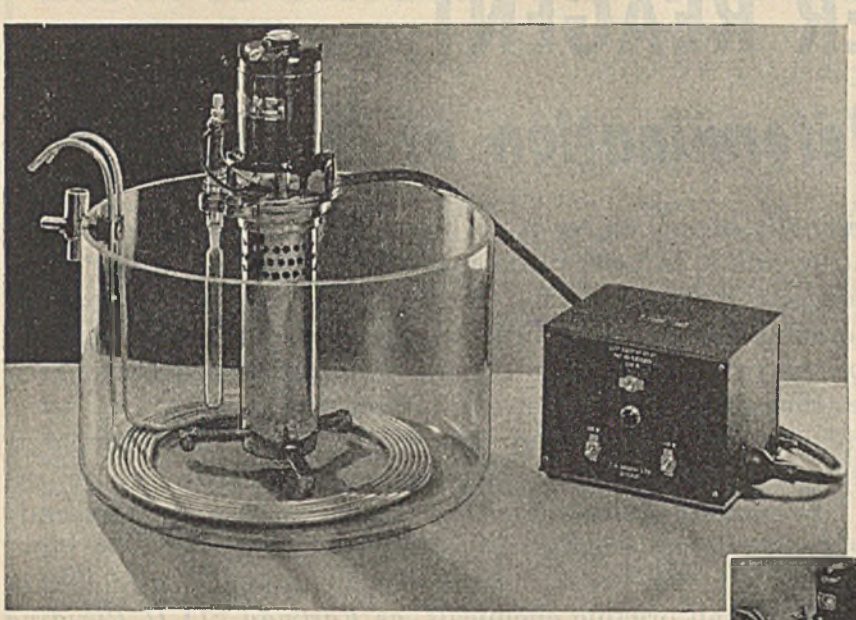
Technique of Assaying Amino Acids

The growing recognition of the importance of the amino acids as nutritional factors has raised the problem of exact assays, comparable to those now applied to vitamins and other such substances in commercial demand.

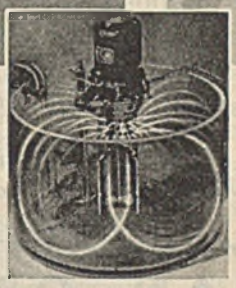
A rapid and practical technique of assay has recently been worked out. After considerable research work, the laboratories of the Wm. T. Thompson Co., 2727 Hyperion Ave., Los Angeles 27, Calif., are in position to supply microbiological assays of the following amino acids:

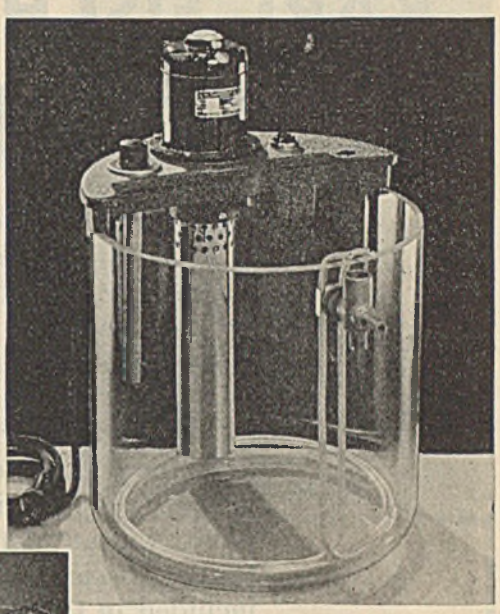
l-(+)-Glutamic acid	*l*-(−)-Cystine	*l*-(−)-Tyrosine
dl-Leucine	*dl*-Threonine	*dl*-Serine
dl-Valine	*dl*-Isoleucine	*dl*-Phenylalanine
l-(−)-Tryptophane	*dl*-Methionine	*l*-(+)-Arginine

CONSTANT TEMPERATURE WATER BATHS



S-84805—0.01°C Constant Temperature Water Bath





S-84860—0.25°C Constant Temperature Water Bath

←Heating-circulating tower contains impeller and heating units. This design provides for efficient circulation, heat transfer and temperature uniformity.

TWO models are available, one provides a temperature uniformity of 0.01°C and the other, a temperature uniformity of 0.25°C. Common to both baths is the circulating and heating system upon which depends their excellent performance and field service record. The heating-circulating system consists essentially of motor, tower, impeller and heating units, all assembled as a single compact unit. By means of this arrangement, heat is correctly applied to the water at the point of its most rapid circulation. As shown above, water is forced up the tower where it passes over the heating elements and is then radially ejected at the top of the tower producing fast, uniform circulation of the entire volume which is necessary for absolute temperature uniformity throughout every area of the bath.

Both baths also provide a high ratio of unobstructed working space to overall size by concentrating control equipment within a small area.

The baths are easy to clean and assemble. The heating-circulating system of each model does not incorporate clamps or supporting devices of any kind. These integral units are merely lifted out of the glass containers.

Immersed vessels are fully visible through the transparent walls of the Pyrex glass containers. Measurements of capillary rise, liquid flow, etc., by cathetometer or by simple unaided observation under water level can be made with undiminished accuracy.

S-84805 0.01°C CONSTANT TEMPERATURE WATER BATH
(Pat. No. 2,037,993)

Consisting of the container, the central circulating and heating unit with motor, the Sargent mercurial thermoregulator, a cooling coil and constant level device, the Sargent Zero Current Relay Unit complete with cord and plug for connection to standard outlet and with armored connecting cable for connection to the water bath. Temperature range, from several degrees above that of the cooling water to about 60°C. Diameter, 16 inches; height, 10 inches; water depth, 9½ inches; temperature uniformity, ±0.01°C. For operation from 115 volt, 60 cycle single phase circuit **$150.00**

S-84815 Ditto. But for 115 volt D.C. circuit..... **155.00**

S-84825 Ditto. But for 230 volt, 60 cycle, single phase circuit **150.00**

S-84835 Ditto. But for 230 volt, D.C. circuit..... **155.00**

S-84860 0.25°C CONSTANT TEMPERATURE WATER BATH

Consists of central circulating and heating unit which includes motor and linear expansion type thermoregulator with temperature reference dial to facilitate selection of temperatures, cooling coil, constant level device, Pyrex glass container, and cord and plug for connection to standard outlet. Height of Pyrex jar, 12 inches; depth, 12 inches; water depth, 11 inches. Motor, thermoregulator, and heating and circulating tower are all conveniently and compactly mounted in a one-piece lightweight casting which rests upon the rim of the glass container. Temperature range, from several degrees above that of the cooling water to about 60°C; temperature uniformity ±0.25°C. For operation from 115 volt, 60 cycle circuits..... **$80.00**

E. H. SARGENT & CO., 155-165 E. Superior St., Chicago, Illinois
Michigan Division: 1959 East Jefferson, Detroit, Michigan

S A R G E N T

SCIENTIFIC LABORATORY SUPPLIES