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INDUSTRIAL AND ENGINEERING CHEMISTRY
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WALTER J. MURPHY, EDITOR

Rapid Determination of Starch

Factors for Starches and Comparison with Acid and Enzymic Hydrolysis Methods

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IN A previous publication (*10*) one of the authors presented a rapid and reasonably accurate method, based on color development with iodine, for the determination of starch in fresh, frozen, and canned vegetables. No comparative data were presented for this method and for accepted enzyme procedures, and although it was recognized that unit weights of starches from various sources yield different intensities of color in iodine solution, data on these differences were not available at the time.

This paper presents factors for the calculation of starch in various vegetables with potato starch as a standard; compares results by the proposed method with those by an enzyme procedure; describes minor revisions that increase the accuracy of the original method; and describes its application to dehydrated foods. Data are presented to show that the enzyme procedure used for comparison is not applicable to all types of starches. For this reason acid hydrolysis was used to establish calculation factors for certain pure starches.

Several thousand samples have been analyzed for starch content by the proposed procedure. These samples included fresh, frozen, dehydrated, and canned vegetables and apples.

PREPARATION OF SAMPLES

If the sample is dehydrated, soak 10 to 20 grams for several hours or overnight, using enough water to keep the material covered. Rehydration may be hastened by heating at 60° C. for 2 hours. Replace any water lost during heating. Add a weight of water equal to the combined weight of the sample and rehydrating water; then disintegrate in an electric food blender. A 100- to 200-gram sample of fresh, frozen, or canned vegetable may be comminuted directly in the disintegrator cup with an equal weight of water. To obtain a more homogeneous product, add approximately half of the water to the sample in the blender and run it at low speed, controlled by a rheostat, until the product is well disintegrated. Then add the remainder of the water and run the blender at maximum speed.

Usually there is little or no difficulty in sampling disintegrated products that have been blanched or heated enough to gel the starch grains. On the other hand, certain raw wet-ground products such as potatoes, which have large starch grains, are difficult to sample because the starch settles out rapidly. This type of material sometimes forms a foam that must be dispersed after blending before a representative sample can be obtained. These difficulties can be minimized by the use of as little water as possible in grinding the samples, so that a thick mixture is obtained. Addition of a few drops of amyl alcohol will disperse the foam.

The percentage of starch in the sample determines the dilutions that should be made to obtain a color with iodine within the range that can be read in the photoelectric colorimeter. The starch content of soybeans, snap beans, and most kinds of squash averages about 1%; that of green peas, 5%; that of lima beans, potatoes, and sweet potatoes, 10 to 20%. Starch concentrations up to 2.5 mg. per final 50 ml. of solution in a colorimeter tube of about 1.25-cm. internal diameter have been found to give color intensities that obey Beer's law. Pea starch gives a color with the iodine solution that is about twice as intense as the color obtained with the same weight of most other starches; therefore the value for the upper limit of the range should be divided by two.

There are two types of plant material for which this method would not serve satisfactorily. One is woody tissue, which cannot be disintegrated by the wet-grinding procedure; the other is any tissue that is very low in starch and for which a dilution of less than 25 ml. after treatment with perchloric acid is necessary.

MODIFICATIONS OF PROCEDURE

Concentrated perchloric acid rapidly hydrolyzes starch to sugar. To minimize the chance of momentary high concentrations of perchloric acid, due to insufficient stirring while it is being added to the sample, it was found advisable to modify the original procedure (*10*). Previously 1 ml. of water was added to a 3-gram sample before the starch was solubilized with 72% perchloric acid. More consistent results were obtained when the perchloric acid was diluted with this water before it was used for solubilization. It is well to make up several hundred milliliters of this diluted acid and cool to remove the heat of dilution before use.

In a detailed study of the effect of variations in solubilizing time on the results of starch analysis, duplicate samples of pea starch, lima bean starch, peas, lima beans, and soybeans were treated for 2, 5, 7, 10, 15, and 30 minutes with perchloric acid. Table I shows that solubilization is complete after 5 to 7 minutes, and that generally the sample may stand in contact with the perchloric acid for 30 minutes with little loss in starch.

Table I. Effect of Variation in Solubilizing Times on Results of Starch Analysis

Treatment with $HClO_4$	Pea Starch Recovered	Lima Bean Starch Recovered	Peas, Starch	Lima Beans, Starch	Soybeans, Starch
Min.	*Mg.*	*Mg.*	%	%	%
2	41	66	3.77	11.4	1.99
5	43	71	3.77	12.8	1.97
7	43	73	4.01	12.5	1.98
10	43	72	3.95	12.9	1.90
15	43	70	3.98	12.9	1.90
30	43	73	3.88	12.8	1.78

Table II. Factors for Converting Results Expressed as Potato Starch Equivalent to True Values

Source of Starch		Factor
Lima beans	Henderson Bush	0.99
	Clark's Bush	1.00
	Fordhook	1.06
Peas	Thomas Laxton	0.53
	Wando	0.53
	Wisconsin Sweet	0.53
	Surprise	0.55
	Tall Alderman	0.55
	Dark "Podded" Thomas Laxton	0.56
	Wisconsin Perfection	0.57
	Wilt-resistant Perfection	0.57
	Laxton's Progress	0.59
	Alaska	0.92
	Early Sweet	1.18
Potatoes	Idaho Russet	0.99
	California White Rose	1.02
Sweet potatoes	Red-skinned	1.20
	Yellow-skinned	1.30
Squash	Buttercup	1.10
	Blue Hubbard	1.12
Wheat	Tenmarq	1.26
Soybeans	Bansei	1.06
Snap beans	Bountiful	1.00
Eucalyptus leaves		1.04

The concentration of the sodium hydroxide used in neutralizing the aliquot before the development of the starch-iodine color was lowered from 6 *N* to 2 *N*, because it was found that prolonged exposure to high alkalinity lowered the values as much as 10% when 5 drops of 6 *N* sodium hydroxide were added in excess of neutrality.

It was found also that excessive salts cause low starch-iodine color values. The results of a study indicated that the solubilized sample should be made up to at least 25 ml., in order to obtain sufficient dilution of the salts.

ESTABLISHMENT OF FACTORS

It is generally recognized that common starches are made up of at least two different compounds (*2*, *8*, *11*), usually designated as amylose and amylopectin. Amylose gives a strong blue color while amylopectin gives a weak violet color with iodine. These properties have been used by McCready and Hassid (*9*) to determine the ratio of amylose to amylopectin in potato starch.

Since starches from various sources have different ratios of amylose to amylopectin, any iodine colorimetric method for the accurate determination of starch must take this fact into account. A unit weight of starch from one source may give a deeper or lighter color with iodine than will starch from another source. This variation does not produce as complex a situation as one might expect, for two reasons. First, the ratio of amylose to amylopectin in the starch of plants appears to be a factor that is inherited and is for all practical purposes the same for different maturities and growing conditions. Secondly, the ratio of amylose to amylopectin in the starch of many plants appears to be similar to that of potato starch, which is about 20% amylose and 80% amylopectin. Two types of starch that vary widely from this value are waxy corn starch, which is practically 100% amylopectin, and garden-type wrinkled-seeded pea starch, which is probably about 75% amylose and 25% amylopectin. All these values are based on the assumption that all amyloses give the same amount of color with iodine per unit weight.

In establishing the factors for various starches, based on pure potato starch as the standard, studies were made on the influence of maturity and of variety of the plants from which the starch was obtained. The pure starch was prepared as previously described (*10*). No attempt was made to remove all the moisture from the starches, since their purity is established by chemical means, and if some moisture is present there is less chance of error in weighing due to absorption of moisture from the air. Any moisture present was therefore regarded as an impurity.

Fifty to 100 mg. of potato starch or *X* starch (any starch other than potato starch) was accurately weighed out; 3 ml. of water were added; the starch was solubilized; and the iodine color was developed and measured as described previously (*10*).

The factors were calculated thus:

$$\text{Factor} = \log \frac{100}{\text{(colorimeter reading of 1 mg. of potato starch in 50 ml.)}}$$

divided by

$$\log \frac{100}{\text{(colorimeter reading of 1 mg. of } X \text{ starch in 50 ml.)}}$$

This formula is used when the colorimeter reading represents percentage of transmission and the instrument is adjusted to read 100 with the reagent blank. If the instrument has a log scale, then the reading corrected for reagent blank for 1 mg. of potato starch per 50 ml. is divided by the corrected reading of 1 mg. of the starch being considered.

With these factors and a curve for pure potato starch, the starch content of any of the vegetable products mentioned can be obtained. The percentage of starch, calculated as potato starch equivalent, is multiplied by the established factor for the product in question. Table II gives the factors for a number of different vegetable starches and for several varieties of certain vegetables. The factors have been related to potato starch because raw potatoes are available throughout the year and because of the ease of preparing pure samples of this starch.

The factors for soybeans, snap beans, and eucalyptus leaves were obtained in a different manner, because of difficulty in obtaining pure starch from them. A starch analysis was made by the proposed method and the result calculated as potato starch equivalent. The same sample was then analyzed for starch by the enzyme procedure (*6*) and the factor was obtained by dividing the percentage of starch obtained with the enzyme procedure by the value obtained for potato starch equivalent.

Sweet corn is the only common starch-storing vegetable that is omitted from the table. The authors were not able to prepare sweet-corn starch that was free from glycogen (*7*) and therefore could not determine the factor for the starch of sweet corn. The

Table III. Effect of Maturity on Starch Factor

Vegetable	Maturity	Factor
Henderson Bush lima beans	Immature	1.01
	Mature	0.98
	Overmature	0.98
Clark's Bush lima beans	Immature	1.02
	More mature	0.98
	Mature	1.02
	Overmature	1.04
Fordhook lima beans	Immature	1.14
	More mature	1.16
	Mature	1.06
	Overmature	1.05
Wisconsin Perfection peas	Mature	0.57
	Overmature	0.58
Wilt-resistant Perfection peas	Immature	0.58
	Overmature	0.55

Table IV. Determination of Purity of Vegetable Starches by Acid Hydrolysis and by Enzyme Hydrolysis

Vegetable	Sample No.	Purity of Starch: Acid hydrolysis, %	Purity of Starch: Enzyme hydrolysis, %
Pea	1	98.1, 98.1	
	2	98.0, 98.2	
	3	94.5, 94.7	
	4	95.3, 95.9	
	5	96.9, 97.5	
Pea, Alaska		97.7, 98.5	90.0, 91.3
Lima bean	1	98.5, 98.5	98.0, 97.9, 97.9, 98.7
	2	92.5, 92.3	92.4, 92.5
	3	98.2, 98.0	99.3, 99.1, 98.6, 99.2
	4	99.0, 98.3, 97.7, 98.8	98.5, 97.8, 98.5, 98.5
Sweet potato (yellow-skinned)		99.2, 97.9	92.7, 94.5, 94.6
Sweet potato (red-skinned)		98.2, 100.0, 98.1, 100.0	94.4, 95.1, 94.5
Potato (white)		94.0, 92.5, 94.7, 93.0, 95.4	93.8, 95.3, 92.8, 93.7 94.1, 93.1

Table V. Comparison of Results of Starch Determinations by Proposed Method and by Enzyme Hydrolysis

Product	Sample No.	Starch Content: Proposed method	Starch Content: Enzyme hydrolysis
		%	%
Peas	1	2.73, 2.71, 2.74	2.05, 2.43
	2	3.76, 3.68, 3.67, 3.68, 3.66, 3.66	2.57, 2.42, 2.55
	3	4.15, 4.17, 4.24	2.55, 1.70, 2.11, 2.44, 2.06, 1.61
	4	2.81	2.15
	5	3.84, 3.85, 3.85, 3.84, 3.95, 3.94, 3.94, 3.95	2.31, 2.50, 2.46
Lima beans	1	15.4, 15.2, 15.1, 15.2, 15.6, 15.6	15.5, 15.5
	2	14.7, 14.7, 14.2	
Soybeans	1	2.00, 1.96, 1.95, 1.84, 1.89	1.84, 1.95
	2	1.41, 1.42, 1.44, 1.40, 1.45, 1.47, 1.42, 1.42	1.31, 1.38
Wheat flour	.	77.5, 77.2, 77.2 77.5	72.5, 79.0, 71.9
Snap beans	.	5.83, 5.85, 5.80 5.83	5.74, 5.88, 5.58

enzyme method for establishing the factor could not be used either, since the starch enzymes hydrolyze glycogen. Brimhall and Hixon (*3*) reported that corn starch has the same amylose content as potato starch. The factor for corn starch would then be 1.00 on the assumption that corn amylose gives the same amount of color per unit weight as does potato amylose. The red color from the glycogen present in sweet corn would not cause nearly so much error in the proposed method as would the sugar from the glycogen by the enzyme procedure.

Table III shows that in most cases the effect of maturity on the starch factor was almost within the range of experimental error, an exception being Fordhook lima beans, in which case the difference between the highest and lowest factors amounted to 10% of the average for the four degrees of maturity.

The purities of the prepared vegetable starches were determined by enzyme hydrolysis (*6*) and by acid hydrolysis (*1*). Enzyme hydrolysis was tried first because this type of procedure is generally used for starch determinations. As a result of difficulties in applying the method to certain starches, the purities were rechecked by the acid hydrolysis procedure. In comparing results obtained by the proposed method with those obtained by the enzyme procedure, difficulties were again encountered with certain types of vegetables.

In the acid hydrolysis 0.1000 gram of starch was weighed out on an analytical balance; 100 ml. of 1 *N* hydrochloric acid were added, and the solution was refluxed 2.5 hours on a hot plate. The solution was then cooled, neutralized to phenolphthalein and made to volume; an aliquot was taken; and sugar was determined by the ferricyanide method (*5*). This procedure was carried out in duplicate or triplicate with very good agreement.

In the enzyme hydrolysis (*6*) 0.5000 gram of the purified starch was refluxed for 30 minutes in acidified alcohol to solubilize the starch. It was then filtered and the alcohol-insoluble residue was taken up in water, boiled 5 minutes, and held on a steam bath for at least 30 minutes. The solution was filtered and the filtrate saved. The process was repeated on the insoluble residue, and the second filtrate was added to the first. The combined filtrates were made to volume; and aliquot was hydrolyzed with salivary amylase for 2 hours at 37° to 40° C.; and the sugar was determined by the ferricyanide method.

To determine the possible presence in the purified starch of certain foreign substances that might raise the sugar titer, photomicrographs of the various starches were prepared. A careful study of these pictures indicated that the starch granules had been separated from the extraneous material.

The authors were not able to obtain consistent results by enzyme hydrolysis on pea starch. The procedure was modified to prevent loss due to handling of the sample and also to solubilize the starch more completely before enzyme hydrolysis. The modifications to prevent loss due to handling did not improve the reproducibility of the results. A longer heating period gave somewhat higher results with pea starch, but even this heating, carried to extremes, did not produce a completely soluble starch. Some pea-starch granules always settled out. The time for enzymatic hydrolysis of pea and potato starches was increased to 6 hours. As a result, the amount of maltose produced was increased by about 7% for peas and by 2% for potatoes.

It can be seen from Table IV that concordant results for all the types of starches investigated were obtained by acid hydrolysis. Enzyme-hydrolysis values were fairly consistent for all of the starches with the exception of starch from peas. The results by enzyme hydrolysis, however, were somewhat lower, except for potato and lima bean starch, than by acid hydrolysis. Because of the lower results obtained by enzyme hydrolysis, acid hydrolysis values were accepted as showing the purities of the starches.

The starch content of several vegetables was determined by the proposed method and by the enzyme hydrolysis method (*6*). In the latter, the sample was dried in a vacuum oven and ground in a Wiley mill, or disintegrated in a Waring Blendor and dried with successive portions of acetone and ether by suction-filtration on a sintered-glass filter. The size of the sample depended upon the range of starch content and the method of preparation. About 0.5 gram of dried material or 3 to 6 grams of wet-ground material was taken. Starch results by the two procedures are shown in Table V. It is evident that the proposed method and enzyme hydrolysis gave fairly comparable and consistent values for snap beans, potatoes, lima beans, soybeans, and wheat flour. The proposed method gave higher values and good duplication for peas, while the enzyme method gave lower and less concordant values. As suggested above, this disagreement may be due to the nature of pea starch.

Empirical factors are used in both methods. The proposed method requires a factor that is dependent on the relative intensity of color developed by iodine per unit weight of starch from a given source. Enzyme methods require factors for the relative extent of hydrolysis of particular starches, and values that are established empirically for the reducing sugars (*5*). From the results obtained it appears that further studies should be made

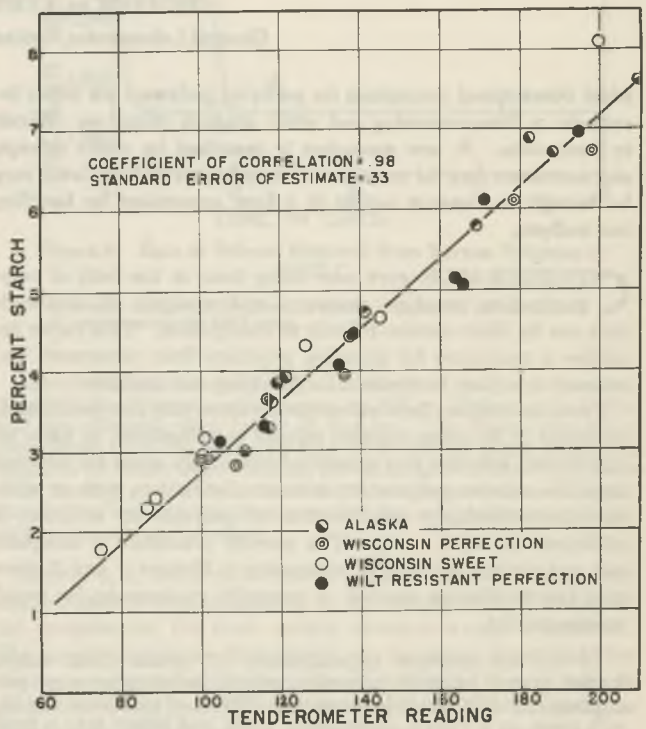

Figure 1. Relation between Tenderometer Readings and Starch Content of Peas

on the enzyme hydrolysis method in order to adapt it to starches, similar to pea starch, which are comparatively high in amylose content.

EFFECT OF AMYLASES IN MATERIAL TO BE ANALYZED

The concentration and activity of amylases in raw vegetable materials vary greatly among different species of plants. In the determination of starch content of a vegetable containing active amylases, special precautions should be observed. If the material must be stored before analysis, it should be frozen and held at −18° C., or, if it is of such nature that it can be heated very rapidly in boiling water to inactivate the enzymes, it may be dried or stored at a temperature low enough to prevent bacterial spoilage. When the determination is made on unheated material containing active amylases, the material should be disintegrated with cold water in a food blender, a sample immediately weighed out, and the perchloric acid added to prevent enzyme action.

Of the materials investigated, including peas, lima beans, soybeans, corn, apples, sweet potatoes, snap beans, carrots, squash, pumpkin, and white potatoes, only the sweet potatoes and carrots appeared to contain very active amylases. It was found, for instance, that when sweet potatoes were diced and held at 66° C. for 5 minutes, about 60% of the starch was converted to maltose. Dropping the diced sweet potatoes directly into boiling water and allowing them to heat for 2 minutes inactivated the amylases to such an extent that only 5 to 10% of the starch was converted to maltose. During storage, the starch in apples is converted to sugar. On the other hand, if apples are disintegrated, an inhibitor is released that prevents the hydrolysis of their starch (*4*). Some evidence was obtained that enzymes in soybeans will convert part of their starch to sugar if they are not heated to the boiling temperature rapidly. From the evidence presented, it can be seen that if a plant material is preserved by drying at 68° C., without quick heating to inactivate the amylases, erroneous starch results may be obtained.

APPLICATION TO GRADING

In a previous publication (*10*) a curve illustrating the relationship between tenderometer readings and starch contents of peas was shown. This curve was drawn from random results obtained on several tenderometers with peas of varied history. Figure 1 shows this relationship when samples of uniform history were measured with one instrument. Under these conditions the correlation coefficient between tenderometer reading and starch content was +0.98. This correlation suggests that starch determinations by the proposed method may be used also in determining maturities on processed peas, whereas the tenderometer can be used only on raw samples.

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Precise Method for Isolation of High Polymers

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Most conventional procedures for purifying polymers are either inaccurate or time-consuming and yield products which are difficult to manipulate. A new procedure is described by which solvents and monomers may be separated from polymers and the latter may be brought to constant weight in a form convenient for handling and analysis.

CONSIDERABLE work now being done in the field of polymerization involves recovery and analysis of materials that are by their nature difficult to manipulate. This paper describes a procedure for isolating polymers from monomers and solvents in a form convenient for handling and analysis.

Previous workers have either precipitated polymer products as a powder (*4, 8*), using suitable solvent combinations, or have assumed that solvents and unreacted monomers could be removed from the massive polymer by vacuum distillation, with or without the preliminary precipitation of polymer or addition of inhibitors (*1, 2, 3, 6, 7*). The powder procedure is adequate but not always possible or convenient; Figures 1 and 2 show that the distillation method is generally inadequate for rapid, precise results.

In the first example approximately 1.5 grams (final weight 1.4866 grams) of high molecular weight polystyrene were precipitated from 20 cc. of benzene with 200 cc. of methanol and the soft mass, in a 125-cc. Erlenmeyer flask, was blown into a froth by a sudden reduction of pressure and then heated at 1-mm. pressure. [Polystyrene used in these experiments was prepared by polymerizing styrene at 60° C. with 0.1 mole % benzoyl peroxide in the absence of air. The styrene was the same as that used in most of the experiments by Mayo and Lewis (*5*). Polymers were precipitated from benzene solution with methanol before use.] The sample continued to lose weight after being heated for a week at 60° C. After the bath temperature was raised to 100°, the sample required an additional 24 hours at 1-mm. pressure to reach constant weight. In the second example, a sample of polystyrene was prepared by the frozen benzene technique, described below, and found to weigh 1.6824 grams.

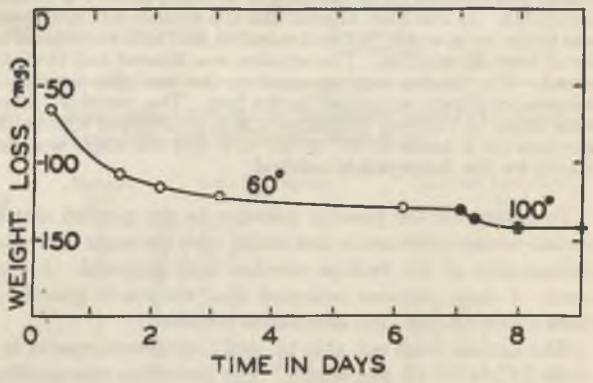

Figure 1. Rate of Solvent Loss from Polystyrene at 60° and 100° C.

Sample, 1.4866 grams, was blown into a coarse froth from benzene.

Without removing the sample from the flask, 20 cc. of benzene were added and the polymer was then precipitated from solution with methanol and treated as in the first example except that the temperature was held at 100° throughout the heating period. *A*, Figure 2, shows that about 50 hours were required to reach a constant weight. This value coincided with the original weight (zero reference line in the graph) within 0.0004 gram.

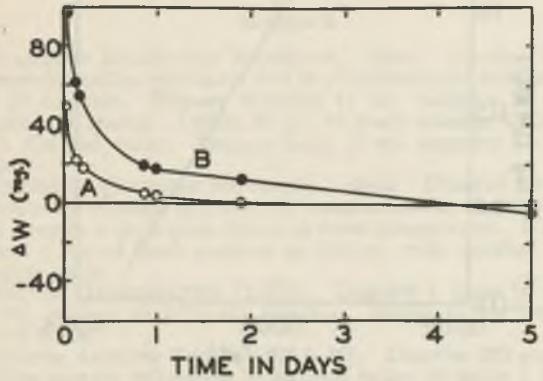

Figure 2. Rate of Solvent Loss from Massive Polystyrene at 100° C.

Polymer was in form of a coarse froth.
A, 1.6824 grams of polystyrene in benzene
B, 1.5325 grams of polystyrene in styrene containing 0.100 gram of hydroquinone

B, Figure 2, records the rate of loss of dissolved monomeric styrene at 100°. A 1.5325-gram sample of polystyrene, prepared by the frozen benzene technique, was dissolved in 20 cc. of styrene, 0.100 gram of hydroquinone added, and the styrene distilled from the flask at reduced pressure. After most of the styrene had distilled off, the weight change was followed at 100° and 1-mm. pressure. The results are plotted in Figure 2, with the reference weight increased by 0.100 gram to allow for the added hydroquinone. After 10 days at 100°, the sample had not yet reached a constant weight; the weight decrease below the reference value is due to partial loss of hydroquinone. Results are variable in such experiments, and, even for a volatile solvent, several days' heating at 100° is often required to attain a weight which remains constant to within 0.3 mg. during a heating period of 2 weeks.

The relatively flat portion of the curve in Figure 1 might easily be mistaken for constant weight, introducing appreciable errors. Further, monomeric styrene is lost more slowly than benzene and

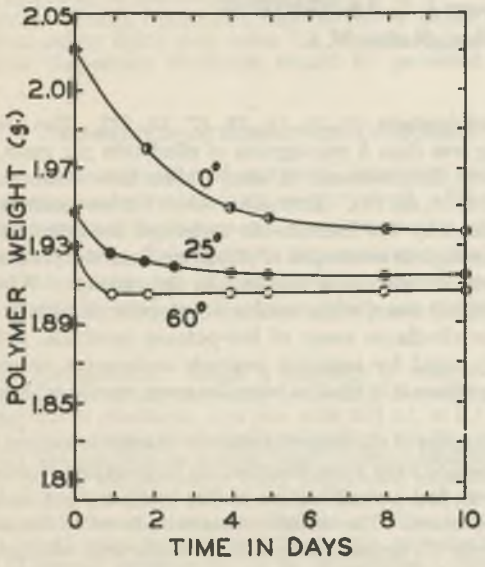

Figure 3. Effect of Temperature on Rate of Solvent Removal from Porous Polymer

At 0°, 25°, and 60° C. Initial polymer weights were not identical.

polymerizes appreciably during the heating period required. Addition of inhibitors introduces uncertainties. Finally, the products of the distillation method are hard, sometimes inhomogeneous masses; besides being difficult to manipulate, different portions of a copolymer sample have given different analytical results.

FROZEN BENZENE TECHNIQUE

The main difficulty in solvent and monomer removal by procedures like that above is that the rate depends on the diffusion of solvent through relatively large masses of hard polymer. This difficulty is largely eliminated by the following technique:

The polymer is dissolved in eight to ten times its weight of benzene and the solution is quickly frozen. The benzene is then sublimed from the polymer without melting of the benzene or sintering of the polymer. The polymer is left as a soft, fluffy, very porous solid, from which remaining traces of volatile materials are rapidly removed, which is easily broken up for transference and analysis and which dissolves readily in solvents.

The experimental data in this paper are based on tests with 2- to 10-gram samples of polystyrene. The authors have employed the procedure in routine treatment of similar samples of other polymers and copolymers which are solid and soluble in benzene, with entirely satisfactory results. Other solvents which are conveniently sublimed could doubtless be employed.

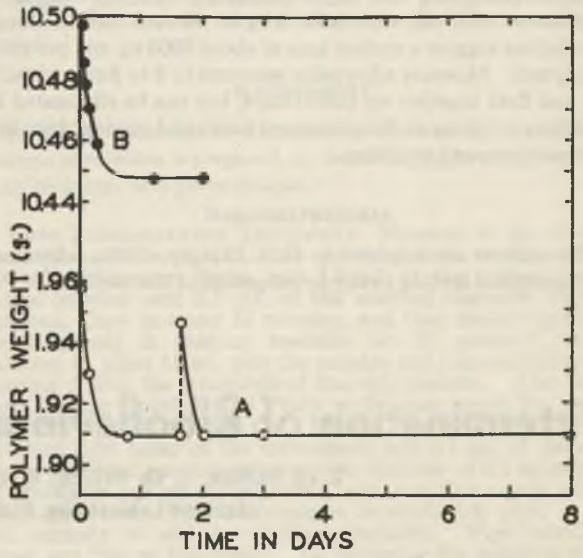

Figure 4. Rate of Solvent Removal from Porous Polymer at 100° C.

A, sample weight 1.9088 grams, polymer redissolved at indicated point
B, sample weight 10.4465 grams

Samples (approximately 2 grams each) of polystyrene were dissolved in 20 grams of benzene in 125-cc. Erlenmeyer flasks and the solutions were frozen in dry ice. The flasks were then transferred to an ice bath and held at 0° C. and 1-mm. pressure. Eight to 10 hours were required for removal of most of the benzene, during which the rate of solvent loss was almost linear. Removal of solvent was then completed at a higher temperature, still under reduced pressure.

Figures 3, 4, and 5 show how the rate at which the polymers attain constant weight depends on the temperature: The higher the temperature, the more quickly constant weight is attained. The upper temperature limit which may be used is determined by the temperature at which the polymer sinters and loses its porous structure. Polystyrene sinters slowly at 100°, the porous cake shrinking to perhaps half its original volume in 10 hours, but no depolymerization has been detected in as long as 2 weeks at 100° and 1-mm. pressure.

A, Figure 4, shows that the results are entirely reproducible. After one sample had been brought to constant weight at 100°, it was redissolved in benzene. Removal of the benzene by the same technique yielded the same weight of polymer. When this polymer was redissolved in benzene and the solvent removed by the distillation technique, the same weight of product was obtained if the heating time was sufficiently long (*A*, Figure 2), showing that moisture or solvent adsorption on the porous polymer was negligible.

The following details should be of assistance in applying the frozen benzene technique.

Pyrex Erlenmeyer flasks have been used because they give fairly large, flat cakes of polymer. Less than 1% of the 125-cc. flasks collapse under vacuum. The less volatile the monomer present, the more important it becomes to reprecipitate the polymer from solution two or more times before the final benzene solution of the polymer is made up. After the benzene has been largely removed at 0°, the product should stand at room temperature for 1 to 3 hours before heat is applied in order to reduce the tendency of remaining small amounts of solvent to cause sintering. *B*, Figure 4, indicates that at 100° a 10-gram sample attained constant weight about as fast as a 2-gram sample. Since the whole procedure is carried out at reduced pressure, no difficulties arise from oxidation or moisture adsorption, although some chlorine-containing products have sometimes lost hydrogen chloride.

Microscopic examination of the unsintered product shows a spongelike structure with interconnecting cells 10 to 50 microns in diameter with cell walls from 5 to 10 microns thick. Rough calculations suggest a surface area of about 2000 sq. cm. per gram of polymer. Moisture adsorption amounts to 2 to 3 mg. on polymer and flask together on humid days, but can be eliminated by admitting dried air to the evacuated flasks and storing them in a desiccator prior to weighing.

ACKNOWLEDGMENT

The authors are indebted to H. C. Tingey of this laboratory for suggestions leading to the development of this method.

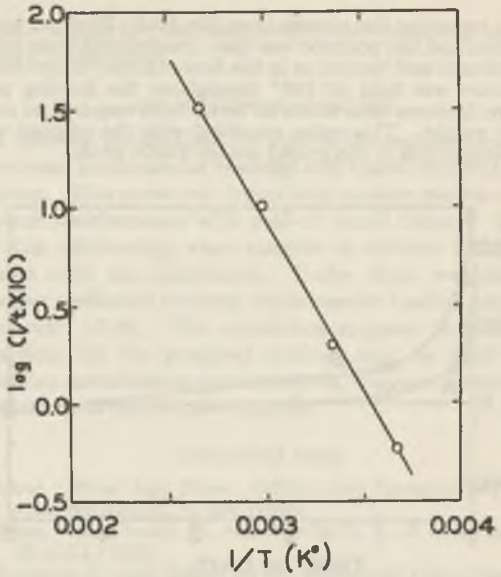

Figure 5. Empirical Relation of Constant Weight Time to Temperature

Times, *t*, in days, required to reach constant weight were 11, 5, 1, and 0.3, respectively, for temperatures of 0°, 25°, 60°, and 100° C., as determined from Figures 3 and 4.

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Determination of Riboflavin in Low-Potency Foods and Feeds

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The method of Hodson and Norris for the fluorometric determination of riboflavin has been modified successfully for use with low-potency materials. The modifications introduced include refinements in the double reduction technique and optional use of both clarase digestion and permanganate oxidation, including a recovery step. The results obtained by this method on a variety of low-potency foods and feeds were in better agreement with microbiological values than were the results of Florisil adsorption methods.

THE need for a rapid, precise method for the determination of riboflavin in low-potency foods and feeds prompted a study of the accuracy of fluorometric procedures (*3*, *6*, *11*) with concomitant comparisons by the microbiological method (*5*, *20*, *21*). In order to obtain accurate measurements of riboflavin consumption by human beings or animals consuming a normal diet of many foods or feeds, a critical examination must be made of natural products possessing low riboflavin contents. In the assay of high-potency products for riboflavin content, fluorometric procedures have been aptly demonstrated to yield values agreeing with the biological assay results obtained with rats, chicks, and bacteria (*9*, *11*, *12*, *13*, *17*, *19*, *23*). For products containing less than 5 micrograms of riboflavin per gram, however, serious disagreements in assay values have often been recorded (*2*, *3*, *13*, *15*, *16*). Erroneous values for low-potency products obtained by the fluorometric technique are largely the results of incomplete extraction of riboflavin from the product and the presence of interfering pigments in the extracts. While the microbiological assay, when conducted properly, is usually satisfactory for riboflavin assay of low-potency products, it is not sufficiently rapid for assaying products undergoing commercial processing where it is vital to have the assay results within a few hours.

The principles of the determination of riboflavin content by an indirect method have been described by Hodson and Norris (*11*). The authors find a modification of this rapid method to be the most satisfactory. The modifications introduced in the present work include (1) extraction in a more dilute acid solution with the aid of a blender; (2) optional use of clarase digestion at pH 4.5; (3) optional use of permanganate treatment at pH 4.5; (4) a riboflavin recovery step when permanganate is used; (5)

double reduction of the riboflavin at pH 4.5 instead of 7 to 7.5; and (6) use of the minimum quantity of sodium hydrosulfite during the second reduction of the riboflavin. Two steps require careful adjustment—the back-titration with dilute hydrogen peroxide after permanganate oxidation, and the second reduction of riboflavin by dilute sodium hydrosulfite.

REAGENTS

Standard Riboflavin Solutions. *Stock.* Dissolve 75 mg. of pure crystalline riboflavin in 0.05 *N* sulfuric acid to make 1000 ml. of solution. Prepare monthly (1 ml. contains 75 micrograms). *Working.* Dilute 20 ml. of stock solution to 100 ml. with distilled water. Prepare daily (1 ml. contains 15 micrograms).

Stannous Chloride Solutions. *Stock.* Dissolve 10 grams of stannous chloride in 25 ml. of concentrated hydrochloric acid and store in a dark glass bottle at room temperature. *Working.* Dilute 1 ml. of stock solution to 500 ml. with distilled water. Prepare daily.

Sodium Hydrosulfite (2.5%). Dissolve 1 gram in 40 ml. of 2% sodium bicarbonate solution. Stable in ice bath for 2 to 4 hours.

Sodium Acetate Solution (2.5 *M*). Dissolve 340 grams of sodium acetate trihydrate in distilled water to make 1 liter of solution.

Permanganate Solution (4%). Dissolve 4 grams of potassium permanganate in distilled water to make 100 ml. Prepare every two weeks.

Hydrogen Peroxide Solution (3%). Dilute 5 ml. of 30% hydrogen peroxide to 50 ml. with distilled water. Store in refrigerator and prepare every two weeks.

Clarase (Takamine Laboratories, Clifton, N. J.). It is desirable to have a preparation of low riboflavin content, less than 5 micrograms per gram. All lots must be assayed.

Sulfuric Acid, approximately 0.1 *N*.

APPARATUS

A fluorophotometer (1) exhibiting sufficient sensitivity to the fluorescence of riboflavin, (2) yielding a linear relationship between galvanometer readings and critical riboflavin concentrations, and (3) demonstrating stability of response during operational period. For general practicability it is desirable to operate the instrument so as to obtain a galvanometer response of 40 to 50 scale divisions for riboflavin concentration of 0.1 microgram per milliliter. The Pfaltz and Bauer fluorophotometer, model B, the Coleman photofluorometer, model 12, the Coleman Universal spectrophotometer, model 11, with UV lamp and 20 by 40 mm. cuvettes, and others are satisfactory.

A Waring Blendor or Wonder-Mix.

A shaking machine with vigorous to-and-fro action.

An autoclave or water bath.

An incubator or low-temperature oven.

Light-protecting equipment, such as red or amber glassware, Eastman safety lights with series OA Wratten light filters. All solutions containing riboflavin should be protected from the light.

PREPARATION OF EXTRACT FOR FLUOROMETRY

All samples may be acid-extracted, incubated with clarase, and permanganate-treated (with recovery step). When in doubt about the value of the clarase or permanganate step, include these in the procedure. It is desirable to use clarase on all products of a starchy nature and on animal tissue. Permanganate treatment should be used on all extracts which are appreciably pigmented.

Acid Extraction. Weigh a quantity of sample (not more than 20 grams of dry material) preferably containing about 35 micrograms of riboflavin, and mix with 150 ml. of 0.1 *N* sulfuric acid. If the sample is not finely pulverized, mix in a blender and transfer the contents to a flask easily accommodating a volume of 250 ml. If the sample is finely pulverized, introduce the dry product into the flask, add the acid solution, and mix by hand-shaking. Either immerse the flask in a boiling water bath or reflux for 45 minutes, and swirl frequently, or autoclave at 6.8-kg. (15-pound) pressure for 15 to 20 minutes. Cool and add 10 ml. of 2.5 *M* sodium acetate solution. If the sample clumps during heating, again macerate in the blender. Dilute to 250 ml. with distilled water and filter through Whatman No. 40 paper.

Enzyme Treatment. If the sample is to undergo enzyme digestion, omit the above dilution and filtration steps, add 3% of the sample weight of clarase (dispersed in the sodium acetate solution), and conduct the digestion for 2 hours at 45° to 50° C. Cool, dilute to 250 ml., and filter. [This filtrate can also be used for thiamine assay by the methods of Hennessy (*10*) or Conner and Straub (*6*).]

Permanganate Oxidation. Measure 50 ml. of the filtered extract, obtained either after acid extraction or enzyme treatment, into each of two 125-ml. clear-glass Erlenmeyer flasks, *U* and *R*. To one, *R*, add 0.5 ml. of riboflavin standard working solution (for recovery purposes). To each flask add the same amount of 4% potassium permanganate solution (2 ml. or more), swirl, and allow to react for 2 minutes. Then add dropwise (titrate) with swirling, just enough 3% hydrogen peroxide solution to cause a disappearance of the permanganate color. This may or may not be accompanied by formation of a precipitate. If a precipitate forms, add hydrogen peroxide only as long as it continues to react, as indicated by progressive diminution of a color due to solution of the precipitate and the fizzing sound caused by evolution of oxygen. Since the reaction is not instantaneous, add hydrogen peroxide carefully, with swirling. If the color is disappearing, stop the addition of hydrogen peroxide when a slight color remains, which will disappear completely after several minutes. If an excess of hydrogen peroxide is suspected, back-titrate dropwise with 4% potassium permanganate until the color of the added permanganate fades slowly. Transfer quantitatively to a 100-ml. glass-stoppered graduated cylinder, add a drop of caprylic alcohol, and dilute to 70 ml. Filter, if necessary, through Whatman No. 42 paper.

For occasional samples, such as some mixed feeds, filtration at pH 4.5 is very slow and may yield cloudy, colored filtrates. In such cases, add 1 *N* sodium phosphate solution, after the hydrogen peroxide treatment, until the precipitate becomes flocculent, dilute to 70 ml., and filter through Whatman No. 42 paper.

FLUOROMETRY

In the preparation of extracts for fluorometry a sufficient volume of solution is prepared, so that a duplicate set of readings may be taken on a given sample.

With Permanganate Treatment. Measure 50 ml. of solutions *U* and *R* obtained after permanganate treatment into 500-ml. Erlenmeyer flasks, add 1.0 ml. of the sodium hydrosulfite solution and 2.5 ml. of the working stannous chloride solution, allow to stand 10 minutes, and then shake vigorously (unstoppered) in shaking machine for 15 minutes. From solution *U*, pipet 15 ml. into the cuvette and take galvanometer reading *A* with the standardized fluorophotometer. (Use 15 ml. for Coleman model 12 and Pfaltz and Bauer model B; use 30 ml. for Coleman model 11.) Protect the cuvette from the incident light beam of the instrument, add 0.1 ml. of the riboflavin standard working solution (the addition of 0.1 microgram of riboflavin per ml. of solution), and mix thoroughly with a small glass rod. (Use a micropipet or serological pipet of 0.1-ml. capacity to add the riboflavin solution. Wipe outside of pipet and "tip to the mark". In delivering the contents of the pipet, place the tip just above the meniscus at the side of the cuvette, allow to drain, then gently blow clean.) Take galvanometer reading *B*. Then add one drop of the sodium hydrosulfite solution, stir, and take blank reading *C*. Add a second drop of sodium hydrosulfite solution to check the completeness of riboflavin reduction. If there is no significant change in the reading upon the addition of a second drop, reduction was complete after the first addition.

From solution *R*, take reading R_1 of a 15-ml. quantity in the cuvette, then reduce the riboflavin by dropwise addition of the sodium hydrosulfite solution and take blank reading R_2.

Without Permanganate Treatment. Measure 50 ml. of the filtrate obtained after acid extraction or enzyme treatment into a 100-ml. graduated cylinder, add 1.0 ml. of the sodium hydrosulfite solution and 2.5 ml. of the stannous chloride working solution, dilute to 75 ml., and allow to stand for 10 minutes. Then pour 45 to 50 ml. into an unstoppered 500-ml. Erlenmeyer flask and shake vigorously for 15 minutes. Take fluorescence readings *A*, *B*, and *C* as described for solution *U*.

CALCULATIONS

W = weight of sample

S = micrograms of standard riboflavin added per ml. of solution = 0.1

V = total ml. of dilution of W = 375

Table I. Effect of Incubation Time with Clarase[a] on Assay Value

Product	Type of Assay	Incubation Time[b] 2 hours	20–24 hours
		Micrograms per gram	
Poultry mash, enriched	Fluorometric	3.4	3.5
Brown rice	Fluorometric	0.42	0.43
Whole-wheat bread	Fluorometric	0.45	0.47
	Microbiological	0.44	0.44
Clarase	Fluorometric	4.2	4.3
	Microbiological	4.3	4.0
Meat extract	Fluorometric	7.8	7.7
	Microbiological	7.6	7.1
Pork loin, fresh	Fluorometric	3.2	3.0
Meat scraps, dry	Fluorometric	4.8	5.3
Meat and bone scraps, dry	Fluorometric	3.7	4.2
Lamb shank, fresh	Fluorometric	1.8	2.0
Beef shank, fresh	Fluorometric	2.4	2.5

[a] 3% of sample weight.
[b] At pH 4.5 and 45–50° C.

Table II. Comparative Assay Results with and without Permanganate Treatment of Extracts

Product	Nonriboflavin Fluorescence[a] With $KMnO_4$	Without $KMnO_4$	Riboflavin Content With $KMnO_4$	Without $KMnO_4$
	%	%	*Micrograms per gram*	
Bread, enriched	42	55	1.86	1.89
Whole-wheat bread	53	72	0.45	0.43
Poultry mash	42	84	2.35	2.47
Poultry mash, enriched	40	70	3.58	3.39
Rice polishings	49	57	1.84	1.79
Brown rice	48	55	0.48	0.47
White rice	51	57	0.29	0.31
Canned beets	70	..[b]	0.14	..[b]
Meat extract	73	..[b]	7.8	..[b]

[a] % nonriboflavin fluorescence is calculated by formula $C/A \times 100$, in which A is fluorometric reading of diluted extract and C fluorometric reading after reduction of riboflavin in extract by sodium hydrosulfite.
[b] Nonriboflavin fluorescence was too great to permit riboflavin evaluation when permanganate was not used.

E = riboflavin content of clarase in micrograms per gram
$0.03\ E$ = clarase blank in micrograms per gram of sample

$$\frac{A - C}{B - A} \times S = \text{micrograms of riboflavin per ml. of sample extract} \quad (1)$$

$$\left(\frac{A - C}{B - A} \times S \times \frac{V}{W}\right) - 0.03\ E = \text{micrograms of riboflavin per gram of sample} \quad (2)$$

The use of the micropipet or serological pipet eliminates the need for volume correction factors for readings B and C. The same amount of riboflavin may be added in a larger volume if the proper volume corrections are applied.

If permanganate treatment is employed, the following index can be used to evaluate the recovery of riboflavin during the permanganate treatment step.

$$\frac{R_1 - R_2}{B - C} \times 100 = \text{permanganate recovery index} \quad (3)$$

A value of approximately 100% indicates no destruction of riboflavin. If the value is less than 90%, riboflavin destruction during permanganate treatment may be suspected, and the assay should be repeated.

EXPERIMENTAL

Other investigators have employed enzymatic hydrolysis in vitamin extraction techniques under varying conditions, with time periods from 0.5 to 24 hours and with concentrations of enzymes from 1 to 15%. Results obtained with an incubation time of 2 hours, as used in the present method, were compared with those obtained with an incubation time of 20 to 24 hours. The results of this comparison, shown in Table I, reveal an occasional increase of about 10% in the value for some meat products, no change for any of the cereals after the longer incubation.

The Hodson and Norris method (*11*), like other fluorometric methods (*8*), has failed when applied to highly colored extracts of low-potency foods and feeds. The authors have found permanganate treatment, which was introduced by Koschara (*14*), generally satisfactory for the removal of interfering pigments without loss of riboflavin. Table II contains a comparison of assay results with and without permanganate treatment. The nonriboflavin fluorescing substances were decreased in every instance by permanganate oxidation. Several deeply pigmented, low-potency products (cf. canned beets and meat extract) could not be assayed by the fluorometric method without bleaching the interfering pigments with permanganate. The permanganate treatment has, however, been made optional in this method because many products give essentially the same riboflavin value within the limits of analytical error, irrespective of whether or not permanganate is used.

The double reduction of riboflavin originally introduced by Hodson and Norris has been continued. Originally this reduction was conducted at pH 7.0 to 7.5, but the authors find pH 4.5 desirable, as no further pH adjustment is usually necessary after that following acid-extraction. Moreover, when the adjustment is made to the higher pH, a precipitate frequently appears, necessitating another filtration at this point. Data presented in Table III demonstrate that identical riboflavin values are obtained whether the double reduction is carried out at pH 4.5 or at 7.0 to 7.5. Furthermore, the nonpermanganate blank is often less at 4.5 than at 7.

Since pigments mask the fluorescence of riboflavin, the question arose as to whether, in a given extract, the fluorometric response would be proportional to riboflavin concentration over the entire probable range of the latter. This was tested by adding progressive increments of riboflavin to the final extract of samples of clarase, whole-wheat bread, and meat extract. As shown in Figure 1, a linear response was obtained in each case, indicating that for these extracts the fluorometric response is proportional to riboflavin concentration.

The recovery of added riboflavin, although it is not an infallible index of the reliability of a method, is of definite assistance in the evaluation of the procedure. Table IV demonstrates that riboflavin added to the sample before extraction or during the determination is recovered quantitatively. The satisfactory recoveries obtained with different levels of riboflavin added to the extract of poultry mash before permanganate treatment illustrate again that the degree of fluorescence is proportional to the concentration of riboflavin.

Table III. Comparative Results with Double Reduction of Riboflavin at Different pH Values

Product	pH 7.0 to 7.5	pH 4.5
	Micrograms per gram	
Poultry mash	2.39	2.43
Rice polishings	1.64	1.66
Brown rice	0.50	0.50
White rice	0.32	0.31
Cookies, enriched	2.10	2.01
Rolls, enriched	1.94	1.96
Bread, enriched	1.83	1.83

The merits of the present method have been evaluated by comparison with the microbiological method (*5*, *20*, *21*) primarily, and also with two commonly used fluorometric methods which include Florisil adsorption, and the methods of Conner and Straub (*6*) and of Andrews (*3*). The microbiological method was conducted according to Snell and Strong (*20*), with the exception that the extracts were prepared so as to avoid growth-stimulating factors described by Bauernfeind, Sotier, and Boruff (*5*) and Strong and Carpenter (*21*). Clarase digestion was employed when warranted. Extracts prepared as described under "Method" have been used satisfactorily for the microbiological assay.

A comparison of typical results obtained by the four methods, presented in Table V, shows that the results obtained by the modified Hodson and Norris method more closely resemble the

microbiological values than do either of the Florisil values. The circumstance of this agreement provides evidence that the values obtained by the present method are closer to the actual riboflavin content of the products analyzed.

The use of Florisil may lead to results which are appreciably higher or lower than microbiological values. The low results may be due to incomplete adsorption and elution of riboflavin from food extracts. It has been indicated in a recent report (*3*) that the adsorptive power of different batches of Florisil may vary and hence cause low results. The batches of Florisil used in the present work gave quantitative recoveries of pure riboflavin up to at least 25 micrograms on a column of the type used by Conner and Straub. Complete recoveries were also obtained when 2.5 micrograms were added to aliquots of food extracts containing about 2.5 micrograms of riboflavin, but poor recoveries were found when 5 to 10 micrograms were added to aliquots of food extracts containing about 5 micrograms of riboflavin. Thus, although some samples of Florisil have been shown to perform satisfactorily for cereal products (*3*, *6*), it would appear necessary to check the recovery of riboflavin for each type of product assayed. In some cases high results were obtained by the Florisil procedures because of the presence of nonriboflavin fluorescent substances in the eluates. The magnitude of this blank cannot always be satisfactorily evaluated by the use of hydrosulfite, nor are these substances completely eliminated in all cases by permanganate treatment. It may, of course, often happen that these opposing factors cancel each other fairly evenly.

Table IV. Recovery of Riboflavin

Product	Content	Added Riboflavin	Recovery
Riboflavin Added to Products before Extraction			
	Micrograms per gram		%
Flour A	1.43	2.20	92
Flour B	0.64	2.20	96
Feed mixture A	2.04	1.10	100
Feed mixture B	2.21	1.10	95
Riboflavin Added to Extract[a] before $KMnO_4$ Treatment			
	Micrograms per ml.		
Rice polishings	..	0.10	102
Brown rice	..	0.10	93
Flour, enriched	..	0.10	100
Wafers	..	0.10	99
Wafer filling	..	0.10	99
Poultry mash	..	0.05	93
	..	0.10	97
	..	0.10	96
	..	0.15	96

[a] Riboflavin content of all extracts was close to 0.1 microgram per ml. before addition of riboflavin for recovery purposes.

Table V. Typical Comparative Results by the Microbiological and Various Fluorometric Procedures

Product	Fluorometric: Andrews	Fluorometric: Conner and Straub	Fluorometric: Present method	Microbiological
	Micrograms per gram			
Poultry mash	1.30	...	2.00	1.93
Poultry mash, enriched	1.81	...	2.9	2.7
Breakfast food A	1.31	1.88	1.46	1.69
B	1.63	2.02	1.38	1.37
C	2.21	2.96	1.29	1.35
D	0.73	1.30	0.94	0.94
E	1.12	1.70	0.61	0.67
Cereal concentrate	4.9	6.0	6.2	6.1
Canned beets	0.060	0.062	0.14	0.14
Dog food	0.76	1.71	2.9	2.5
Clarase A	2.6	3.5	4.6	4.7
Clarase B	2.0	2.8	4.9	4.3
Lamb shank, fresh	1.26	1.34	1.90	1.87
Beef shank, fresh	1.77	1.83	2.44	2.22
Bread, enriched[a]	2.7	3.0	3.1	3.0
Flour, enriched[b]	2.1	2.3	2.7	2.7
Flour, unenriched	0.06	0.08	0.32	0.28
Brown rice	0.30	0.48	0.47	0.65
White rice	0.22	0.31	0.28	0.42

[a] Collaborative sample; average value reported to be 3.0 (*1*).
[b] Collaborative sample; average value reported to be 2.8 (*1*).

Table VI. Typical Comparative Assays of a Variety of Foods and Feeds

Product	Present Method	Microbiological	Ratio[a]
	Micrograms per gram		%
Bread, enriched	2.08	2.10	99
Cracked-wheat bread	1.76	1.68	105
Whole-wheat bread	0.95	0.93	102
Flour, enriched	3.04	3.00	101
Chocolate, enriched	4.8	4.8	100
Cake, enriched	1.59	1.50	106
Wafers	2.65	2.80	95
Wafer filling	3.92	3.73	105
Whole-wheat, ground	0.86	0.84	102
Whole-wheat flour	0.96	0.88	109
Wheat bran	2.74	2.50	110
Meat and bone scraps, dry	3.7	3.9	95
Soybean oil meal, solvent	3.4	2.9	117
			Av. 104

[a] $\frac{\text{Fluorometric value}}{\text{Microbiological value}} \times 100$.

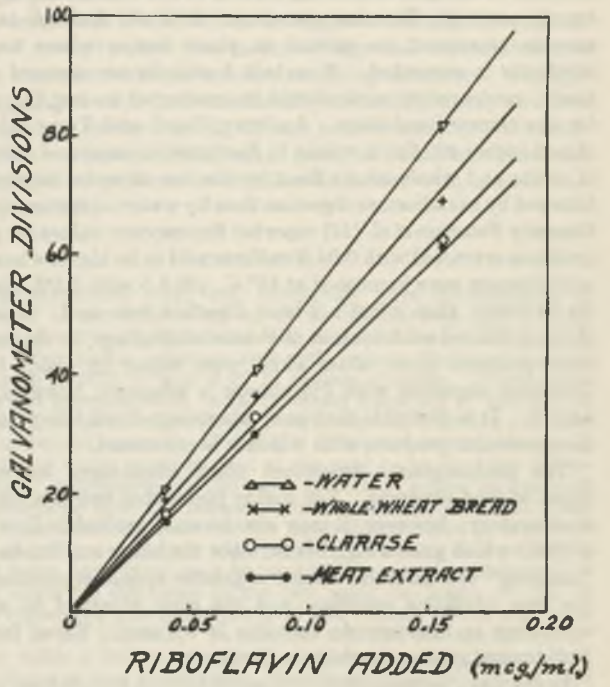

Figure 1. Relation of Fluorescence to Riboflavin Concentration

Table VI shows further representative data comparing results obtained by the microbiological and the present method, including products which were not evaluated by the Florisil methods. For the large variety of materials analyzed, the agreement is, on the whole, satisfactory. The outstanding exceptions are the results on brown and white rice, which are distinctly lower by all three fluorometric methods (Table V). Further work on rice is in progress. Results with samples of mixed feeds occasionally were somewhat higher by the present method. Since better recoveries of added riboflavin have been obtained by the fluorometric method, results obtained with mixed feeds have been judged to be more reliable by the fluorometric than by the microbiological method.

DISCUSSION

In analyzing a wide variety of foods and feeds, one encounters varying extraction problems, such as the choice of suitable solvents, subdivision of the sample, etc. The extractant used, 0.1 *N* sulfuric acid, is believed to be sufficiently strong. Extraction with a 1 *N* acid solution has failed to show any increase over values obtained with 0.1 *N* acid. On the other hand, the use of acid ex-

traction has been reported to yield somewhat higher values than water extraction (*3*). Proper maceration or pulverization of the sample is an important feature of the extraction technique and hence it is recommended that a grinding mill or/and blender be used freely. On some feed samples, maceration of the sample for a few minutes in the acid extraction and enzyme treatment process has resulted in a 50% increase in the riboflavin value. It is further believed that proper maceration of the sample shortens the time necessary for digestion with clarase.

Clarase is an enzyme complex containing amylase as its chief constituent, as well as a number of other enzymes: maltase, inulase, lactase, dextrinase, catalase, various proteases, peptidase, lipase, phosphatase, invertase, and other enzymes in small amounts. It is used in the thiochrome method (*6*) and has been found by Peterson, Brady, and Shaw (*18*) to be more satisfactory in the riboflavin fluorometric method than other enzymes when tested under their conditions. In the fluorometric method the authors find enzyme digestion desirable when extracting large amounts of samples containing starch, as the liquefaction of the starch speeds the filtration procedure. It is also desirable to use enzyme treatment on animal or plant tissues where bound riboflavin is suspected. If certain foodstuffs are assayed routinely, comparative trials should be conducted to test the need for the clarase incubation. Andrews, Boyd, and Terry (*4*) obtained higher riboflavin values in fluorometric assays on samples of white and whole-wheat flour by the use of water extraction followed by takadiastase digestion than by water extraction alone. Recently Peterson *et al.* (*18*) reported fluorometric values for pork products extracted with 0.04 *N* sulfuric acid to be higher when the acid extracts were incubated at 45° C., pH 4.5 with 2.5% clarase for 24 hours, than when a 2-hour digestion was used. Table I shows a limited confirmation of Peterson's findings, in that some meat products gave values which were higher by about 10%. Two-hour digestion with 3% clarase is adequate, however, for cereals. It is desirable that each laboratory check this point on the particular products with which it is concerned.

The permanganate treatment offers advantages for many types of food products. For highly pigmented extracts, its use is obligatory; however, it may also be used profitably for other extracts which gave a high blank, since the blank and fluorescent "masking" are reduced. Hence a greater spread is obtained in the true riboflavin readings, and the error attached to minor variations in fluorometric response is reduced. These factors tend toward greater precision in the assay.

In the past, permanganate oxidation has not always been satisfactory, because the amount of permanganate was fixed or because the subsequent addition of peroxide interfered with the hydrosulfite blank. In the present method, neither the amount of permanganate nor the time of reaction need be considered as fixed—for example, the beet extract was so intensely colored that 5 ml. of permanganate were used instead of the usual 2 ml. The other objection, interference of excess peroxide with the hydrosulfite blank, is overcome by the circumstance that any excess peroxide is completely destroyed by the large amount of hydrosulfite used in the first stage of the double reduction.

The incorporation of the recovery step provides a check on possible destruction of riboflavin by permanganate. This occurs only when excessive quantities of permanganate are used and is an unusual event. Occasionally a precipitate will form upon the addition of the permanganate solution. Often this precipitate will dissolve again as the peroxide solution is added. If a significant precipitate remains, it should be filtered off; however, if it is only a slight haze, filtration is not necessary. Hodson and Norris (*11*) have reported (and the authors can confirm) that a faint cloudiness does not interfere seriously with the fluorometric measurements.

Swaminathan (*22*) has criticized the usefulness of the double reduction procedure, as originally presented by Hodson and Norris. Without offering any supporting experimental evidence, he states that "repeated attempts by the author to use their technique have not proved successful, since it was found that the interfering blue fluorescent materials behaved in the same manner as riboflavin". Careful examination of his paper does not reveal reference to the use of a filter between the cuvette and the photocell. The absence of a fluorescent light filter would lead to the measurement of nonspecific fluorescence. Modern fluorometers are provided with a filter which absorbs wave lengths lower than the yellowish-green fluorescence of riboflavin. Swaminathan's criticism therefore does not apply to the present method.

SUMMARY

The fluorometric method of Hodson and Norris for the determination of riboflavin has been adapted to low-potency products. Interfering colored pigments occurring in some of the products have been eliminated or reduced by the use of a controlled permanganate oxidation treatment, which also includes a recovery step. Results by this method showed better agreement with the microbiological assay than did results by fluorometric procedures employing Florisil adsorption.

ACKNOWLEDGMENT

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ADDENDUM

After this paper had been submitted for publication, Daniel and Norris (*7*) presented comparative fluorometric and microbiological data on the riboflavin content of milk and milk products, utilizing a modification of the Hodson and Norris method which is similar to that described here. Daniel and Norris likewise obtained very satisfactory agreement between the two types of assays.

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Modified Methyl Yellow Indicator

For Direct Titration of Sodium Carbonate

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THE peculiar difficulties associated with the titration of sodium carbonate with hydrochloric acid are due in the main to the pronounced buffer effect of the solution on either side of the end point. This buffer effect decreases markedly the response of the indicator to additions of acid, which correspondingly decreases the precision with which one may determine the end point. The end point occurs in the middle of a "transformation interval" throughout which the color change is gradual and, consequently, is not distinguished by a sudden change in color and may not possess a distinctive hue that may be reproduced precisely without comparison with a standard of reference.

Although the use of modified indicators apparently has not become common practice, it has long been known (*4*, *5*, *7*) that more satisfactory results may often be obtained with such an indicator. The modified indicator possesses two advantages. In the first place the modifying colored substance may filter out a portion of those wave lengths that are not appreciably absorbed by either the acid form or the base form of the indicator. Thus, the change occurring at the end point is made more apparent to the eye. Again, the possibilities of varying the relative amounts of indicator and modifying substance permit production of an indicator that at the end point may have a distinctive or familiar hue which may be more easily reproducible than that resulting from the indicator alone.

Several modified (*2*, *8*) or mixed (*3*) indicators have been suggested for use with the titration of sodium carbonate. The author's only justification for suggesting another is that he has found the one herein described more satisfactory for precise work.

As indicator for this work methyl yellow was selected rather than the more commonly used methyl orange because the former was found to give more easily determinable color changes at the end point. Absorption curves for the two forms of methyl orange and methyl yellow and for methylene blue are shown in Figure 1. Data for the curves were obtained with a Coleman Universal spectrophotometer. Those marked methyl yellow (acid) and methyl orange (acid) were obtained in solutions buffered at pH 2.2; those marked methyl yellow (base), methyl orange (base), and methylene blue, in solutions buffered at pH 7.0. These curves compare favorably with those obtained by Mellon and Fortune (*6*); however, they are presented here only for comparative purposes.

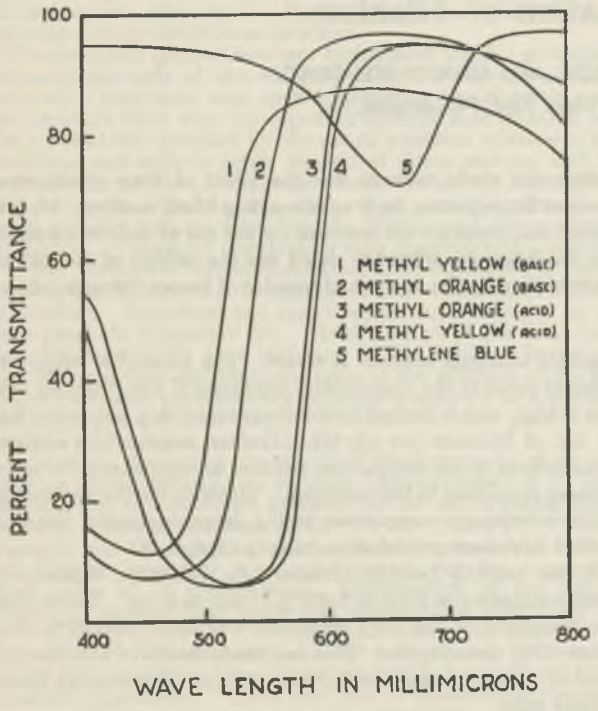

Figure 1. Absorption Curves for Methyl Orange, Methyl Yellow, and Methylene Blue

A comparison of the curves in Figure 1 indicates the superiority of methyl yellow over methyl orange and the suitability of using methylene blue as a color-modifying substance. Methylene blue has been found satisfactorily to modify such yellow-red indicators as methyl red (*2*, *5*) and neutral red (*5*), while Kolthoff (*5*) has reported that equal parts of methylene blue and methyl yellow produce an excellent indicator with transformation point 3.3.

EXPERIMENTAL

Transformation point and sensitivity were investigated on mixtures of alcoholic solutions of methylene blue and methyl yellow of varying proportions and concentrations. These were made using buffer solutions and with carbon dioxide–saturated solutions of 0.25 *N* and 0.10 *N* sodium chloride. These mixtures are identical with those obtained when 0.5 *N* and 0.2 *N* hydrochloric acid, respectively, constitutes the titrating acid and an equivalent quantity of sodium carbonate is dissolved in a volume of water equal to that of the acid to be used for the titration. A proportion of 20 parts of methyl yellow to one part of methylene blue was found to yield an indicator with a sensitive transformation point at the equivalence point of the titration of sodium carbonate with 0.2 *N* hydrochloric acid at 20° C. This same proportion may be used for titrations involving 0.5 *N* acid, since the difference in the end point in terms of 0.5 *N* acid represents less than 0.01% of the volume of the acid consumed in the titration. In alkaline solution the modified indicator yields a yellow-green hue; as the end point is approached this changes to a straw color, then to a sunburned straw, and finally to a pink straw at the end point. A concentration of 0.8 gram of methyl yellow and 0.04 gram of methylene blue per liter of alcohol was found to be suitable. Approximately 0.1 ml. of this mixture is used per 100 ml. of solution contained in the titration flask at the end point. The end point is taken as the point at which the first trace of pink is observed in the solution.

During the course of this work it was noted that the methyl yellow indicator fades rapidly in alkaline solution. The effect of this upon the end point is appreciable if the indicator is added before the titration is started. This effect may be eliminated by deferring addition of the indicator until the titration is more than half completed.

To determine the precision obtainable when the mixed indicator described above is used, several 100-ml. portions of 0.1 *N* solution of sodium chloride were kept saturated with carbon dioxide at 20° C. and titrated back and forth with standard acid and base from the basic side to the first appearance of pink in the solution. The results obtained indicated that as far as the sensitivity of the indicator is concerned the precision obtainable is 0.04% with 0.2 *N* acid and 0.02% with 0.5 *N* acid. This indicates the possibility of obtaining a precision better than that obtained previously by the direct method. To test the indicator adequately, therefore, it was necessary to use methods which would ensure a precision of at least 1 part in 5000.

Table I. Corrections for Carbon Dioxide Unsaturation and for Temperature Deviations

	Normality of HCl	
	0.2	0.5
Unsaturation correction, ml. of HCl	−0.02	0.00
Correction for temperature deviation, HCl % per °C.	−0.01	−0.004

Table II. Direct Titration of Sodium Carbonate with Hydrochloric Acid, Using Modified Methyl Yellow Indicator

(Hydrochloric acid, prepared concentration, 0.2113)

Run	Weight of Na_2CO_3	Weight of Acid	Equivalent of HCl per 1000 Grams
	Gram	*Grams*	
1	0.7004	62.53	0.2113
2	0.5024	44.88	0.2112
3	0.6189	55.27	0.2113
4	0.6621	59.12	0.2113
5	0.5628	50.27	0.2112

Deviations common to the direct titration of sodium carbonate are those ordinarily associated with the use of volume burets, those caused by the fact that the end-point solution may not be saturated with carbon dioxide at atmospheric pressure, and those caused by the variation of the end-point color with temperature.

To ensure a suitable precision in mechanical operations weight burets were used. The values of "unsaturation correction" and the correction for the deviation of the end-point color with temperature were determined for a particular set of titration conditions. Under the conditions specified and over a wide range of sample size and titration speed, the "unsaturation correction" was nil when 0.5 *N* hydrochloric acid was used and remarkably constant and reproducible when 0.2 *N* acid was used. The correction for temperature deviations from 20° is not large but becomes significant when temperature variations as large as 5° or 6° are encountered. Results are summarized in Table I. The titration vessel was a 250-ml. wide-mouthed titrating flask. The sample was dissolved in a volume of water approximating that of the acid required for the titration. The indicator was added when the titration was more than half completed.

The direct titration of samples of sodium carbonate was carried out under the specified conditions. The hydrochloric acid was made up from constant-boiling acid prepared by the method of Foulk and Hollingsworth (*1*). The sodium carbonate was "volumetric standard" material obtained from a well-known firm. The factor of purity of this salt was determined by comparison with standard hydrochloric acid by the indirect method. Results of the direct titration are given in Table II. The weights of sodium carbonate shown have been corrected for the factor of purity (0.9998) and the weights of hydrochloric acid have been corrected for carbon dioxide "unsaturation" and for deviations of the temperature of the end-point solution from 20° C. The results indicate that the modified methyl yellow indicator is suitable for precise titration of sodium carbonate with 0.2 *N* hydrochloric acid.

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Application of Colorimetry to the Analysis of Corrosion-Resistant Steels

Photoelectric Determination of Titanium

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A method using hydrogen peroxide for the determination of traces of titanium in corrosion-resistant steel has been developed and adapted to the photoelectric colorimeter. Titanium cannot be completely separated from large amounts of iron, chromium, and nickel by the use of a single cupferron precipitation. A spectrophotometric study suggests that the effect of these interferences can best be overcome by a compensating blank reading. Molybdenum and vanadium are removed by the use of sodium peroxide. The procedure is outlined in detail and the validity of the method is established by the analysis of samples of known titanium content.

DURING the past year this laboratory has conducted an extensive investigation of methods for the determination of residual elements in corrosion-resistant steels. As a result, a number of accurate and precise colorimetric methods have been developed for the determination of certain elements at low residual levels.

This publication represents the first of these methods. Others will be presented later, as sufficient analytical data become available.

PHOTOELECTRIC DETERMINATION OF TITANIUM

Small amounts of titanium are commonly determined by the colorimetric procedure using hydrogen peroxide. In this procedure, the titanium is separated from interfering elements and isolated in a sulfuric acid solution. Hydrogen peroxide is then added, and the resultant color is matched by a blank to which standard titanium solution is added. The method is subject to personal error in the comparison, especially if the titanium content is high, and is limited to solutions containing not more than 0.1 mg. of titanium per ml. (*3*). It often requires the addition of impurities to the comparison solution to compensate for interferences remaining in the unknown. Methods for the application of the colorimetric comparison to the determination of titanium in steel have been published previously (*3*, *6*, *7*, *9*).

It has recently become necessary to determine titanium in steels containing as little as 0.001% of this element. These steels are frequently of the 19% chromium–9% nickel and 25% chromium–20% nickel types. This has made desirable the development of a more accurate and precise method of estimating the titanium color.

The photoelectric colorimeter has been used for the determination of titanium, but application to the analysis of corrosion-

resistant steel has been limited. Bendig and Hirschmüller (*1*), in their study of the photoelectric measurement of the titanium complex, considered the interfering effect of ferric ion only. The method of Sokolova (*8*) for the photoelectric determination of titanium was not applicable to steels high in chromium. Chemical separation of the interfering elements is not entirely satisfactory, since significant amounts of impurities may remain, especially in highly alloyed corrosion-resistant steels. It has been suggested that the effect of residual interferences be nullified by setting the zero point of the colorimeter on a companion sample run simultaneously (*12*). This has the disadvantage of necessitating two determinations, as well as presupposing equal interference in both cases. Kenigstul (*5*) has determined titanium in cast iron, without preliminary separations, by setting the blank with a portion of the solution prior to the peroxidation. However, the effect of molybdenum and vanadium, which react with hydrogen peroxide, was not discussed. Boyer (*2*) has used a similar direct method for the analysis of titanium-bearing corrosion-resistant steel, but vanadium and columbium interfere to varying degrees. The authors have found the method unsatisfactory for extremely low percentages of titanium. Thomas (*10*) has separated chromium by volatilization as chromyl chloride, but the procedure has disadvantages similar to those of Boyer's method.

In the proposed method, most of the interferences are removed by preliminary separations. Remaining impurities are stabilized to ensure reaction of only titanium upon the addition of hydrogen peroxide. The principle of a compensating blank colorimeter reading is then utilized for the photoelectric measurement of the titanium color.

EXPERIMENTAL

Portions of a standard titanium sulfate solution, prepared from recrystallized potassium titanium oxalate (*11*) and containing the equivalent of 0.05 to 2.5 mg. of titanium per 100 ml. of solution, were treated with sulfuric acid and hydrogen peroxide. The resultant color was measured in a Klett-Summerson (test-tube model) photoelectric colorimeter, which has a logarithmic scale. A Klett-Summerson No. 42 (blue) filter was used. The scale readings were found to be directly proportional to the titanium content, showing that Beer's law applied within the concentration range under consideration.

Titanium-free high-chromium high-nickel steels, containing measured amounts of the above titanium solution, were then analyzed. Cupferron was used to separate the titanium, and the peroxide color was developed in sulfuric acid solution (*3*). The method was modified by the use of a solvent mixture of hydrochloric and sulfuric acids, instead of dilute sulfuric acid, to facilitate the solution of high-alloy steels. This was followed by evaporation to dehydrate the silica, thus permitting the use of larger samples without retarding the filtration of the cupferron precipitate. The moist precipitates were digested with a mixture of nitric, perchloric, and sulfuric acids and evaporated to fumes of sulfuric acid, instead of being ignited and fused with potassium pyrosulfate. Vanadium and molybdenum were removed by sodium peroxide separation (*6*). Measurement of the final color photocolorimetrically gave high and erratic results. Investigation disclosed that the solutions, prior to the addition of the hydrogen peroxide, gave positive readings compared to a sulfuric acid–hydrogen peroxide blank. Qualitative tests proved the presence of chromium, nickel, and iron.

In order to determine the extent of interference by these elements, a dilute sulfuric acid solution (approximately 4 *N*) containing 5 mg. of peroxidized titanium per 100 ml. of solution was prepared. Similar solutions of Cr^{+++}, Ni^{++}, and Fe^{+++} were prepared, and the transmission of each was measured by a General Electric recording spectrophotometer set for a spectral band width of 10 millimicrons. Examination of the curves (Figure 1) showed that there was considerable absorption by Cr^{+++}, Ni^{++}, and Fe^{+++} in the region below 440 millimicrons (No. 42 filter). Between 480 and 500 millimicrons, the absorption by Ni^{++} and Fe^{+++} was almost negligible, but that of Cr^{+++} remained appreciable. There was also a considerable decrease in absorption by titanium complex at the higher wave length. For these reasons, it was believed that attempts to eliminate the interference by use of a filter having a mean transmission of about 490 millimicrons would prove unsuccessful. This was

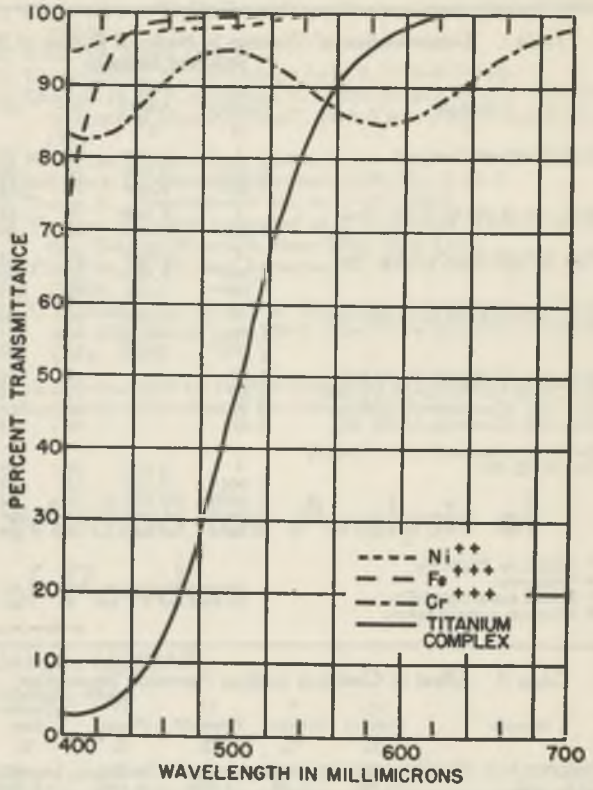

Figure 1. Transmission Curves of Nickel, Ferric, Chromic Sulfates, and Titanium Complex

confirmed by several determinations. Accordingly, all subsequent measurements were made in the region of maximum titanium sensitivity.

It was decided to use the compensation method of correcting for the interferences. This required an initial colorimeter reading before the peroxide addition to measure the absorption caused by residual impurities. Since the chromium was present as $Cr_2O_7^{--}$ prior to the peroxidation, this could not be done directly. The chromium was therefore first reduced with sulfurous acid, the excess sulfur dioxide removed by boiling, and the ferrous ion reoxidized with nitric acid. This treatment stabilized the interfering colors, and the initial reading was taken. After development of the titanium color, a second reading was taken. The titanium absorption was represented by the difference between the two readings. The actual percentage present was calculated from the conversion factor predetermined for the instrument. The results of the analysis of steels of known titanium content are given in Table I.

In order to determine the extent to which molybdenum and vanadium would interfere in the colorimetric measurement of titanium if the sodium peroxide separation were omitted, samples containing varying percentages of these elements were analyzed. The results of this series of determinations are given in Table II.

REAGENTS

Solvent Acid Mixture. Dilute 310 ml. of concentrated hydrochloric acid with 595 ml. of water. Stir continuously while adding 95 ml. of concentrated sulfuric acid.

Cupferron Solution. Dissolve 6 grams of cupferron (ammonium salt of *N*-nitroso-*N*-phenylhydroxylamine) in 100 ml. of water. The solution should be freshly prepared and filtered before using. To prevent decomposition of the solid reagent, keep a small sack of lump ammonium carbonate in the container.

Sodium Hydroxide Solution (25%). Dissolve 250 grams of sodium hydroxide in water and dilute to 1 liter.

Standard Titanium Solution. Transfer 3.68 grams of potassium titanium oxalate [$K_2TiO(C_2O_4).2H_2O$], which has been purified by recrystallizing the salt once from water and drying at room temperature for several days, to a 500-ml. Kjeldahl flask. Add 8 grams of ammonium sulfate and treat with 100 ml. of concentrated sulfuric acid. Gradually heat to boiling and boil for 5 to 10 minutes. Cool somewhat, dilute to 800 ml., and transfer

Table I. Determination of Titanium in National Bureau of Standards and Laboratory Standard Samples

Sample	Ti Present %	Ti Added %[a]	R_1	R_2	R	Ti Found %	Deviation %
N.B.S. 55a (ingot iron)	[b]	0.000	18	19	1	0.000	...
	0.000[c]	0.010	24	63	39	0.010	±0.000
	0.000[c]	0.020	28	108	80	0.021	+0.001
N.B.S. 135 (5.2% Cr–0.6% Mo)	[b]	0.000	17	21	4	0.001	...
	0.001[c]	0.005	24	49	25	0.006	±0.000
N.B.S. 133 (13.6% Cr–0.6% Mo)	[b]	0.000	24	25	1	0.000	...
	0.000[c]	0.005	23	39	16	0.004	−0.001
	0.000[c]	0.021	42	124	82	0.021	±0.000
N.B.S. 101b (18.5% Cr–9.0% Ni)	[b]	0.000	29	30	1	0.000	...
	0.000[c]	0.020	53	131	78	0.020	±0.000
	0.000[c]	0.050	44	236	192	0.050	±0.000
N.B.S. 121 (17.8% Cr–9.0% Ni)[d]	0.39	...	12	159	147	0.39	±0.00
N.B.S. 121a (18.7% Cr–10.6% Ni)[d]	0.35	...	14	146	132	0.35	±0.00
N.B.S. 107 (Cast iron–0.7% Mo)	0.037	...	19	161	142	0.037	±0.00
N.B.S. 115 (Cast iron–15.9% Ni)	0.021	...	49	142	93	0.024	+0.0003
Laboratory standard 24/19 (24.1% Cr–19.2% Ni)	[b]	0.000	24	29	5	0.001	...
	0.001[c]	0.005	25	47	22	0.006	±0.000
	0.001[c]	0.010	22	63	41	0.011	±0.000
	0.001[c]	0.025	28	126	98	0.025	−0.001
	0.001[c]	0.040	29	176	147	0.039	−0.002
						Av. deviation	±0.0006

[a] Added as $Ti_2(SO_4)_3$.
[b] Unknown.
[c] Found experimentally.
[d] 0.5-gram sample taken.

Table II. Effect of Omitting Sodium Peroxide Separation

Sample	Mo Present %	V Present %	Ti Present[a] %	Ti Found %	Deviation %
N.B.S. 73a	0.07	0.03	0.000	0.001	+0.001
N.B.S. 101b	0.08	0.05	0.000	0.002	+0.002
Laboratory standard 24/19	0.04	0.04	0.001	0.002	+0.001
Laboratory standard 2–72/3–55	0.09	0.00	0.000	0.001	+0.001
Laboratory standard 1–72/4–55	0.04	0.00	0.000	0.000	±0.000
Laboratory standard T-3037	0.01	0.08	0.000	0.004	+0.004
Synthetic standard, 2.5 grams T-3037 + 2.5 grams 55a	0.005	0.04	0.000	0.002	+0.002

[a] As determined by procedure given using separation for molybdenum and vanadium.

to a 1000-ml. volumetric flask. Dilute to the mark and filter into a glass-stoppered bottle. Each milliliter of the solution will contain approximately 0.5 mg. of titanium. Check the strength of the solution by taking several 100-ml. portions, diluting to 250 ml., boiling, and precipitating with ammonium hydroxide and boiling for a minute. Filter, swab, and rinse well with hot water. Ignite and weigh as TiO_2.

METHOD

Dissolve a 5-gram sample (for a titanium content of less than 0.05%), in 80 ml. of solvent acid mixture in a 250-ml. beaker by warming gently. Evaporate almost to dryness, but do not bake. (Bringing to low volume on the hot plate, with the use of a sand bath toward the end of the evaporation to avoid spattering, is rapid and effective.) Dilute to 150 ml. with hot water and stir until soluble salts are dissolved. Cool in a running water bath to below room temperature.

Add a ball of filter paper pulp about 1.25 cm. (0.5 in.) in diameter, stir well, then add cooled cupferron solution dropwise, with constant stirring, until the precipitate assumes a deep reddish-brown color (3 to 5 ml. are generally required). Stir for 2 or 3 minutes and allow to settle in the water bath for a few minutes.

Filter on a fine filter paper containing about half the above amount of paper pulp, rinse several times, and wash 10 times with cold diluted sulfuric acid (5 to 95). Transfer the paper and the precipitate to the original beaker, add 25 ml. of concentrated nitric acid, then 10 ml. of concentrated sulfuric acid and 10 ml. of perchloric acid (70%). Destroy the organic matter by evaporating to fumes of sulfuric acid. Cool, cautiously dilute to 75 ml., and warm to dissolve soluble salts. Cool, and add sodium hydroxide solution (25%) until the solution is just alkaline. Add 0.5 gram of sodium peroxide in small portions, stirring vigorously after each addition. When the reaction has subsided, heat to boiling and continue to boil for at least 10 minutes.

Filter through a coarse filter paper to which a little pulp has been added, rinse, and wash with diluted ammonium hydroxide (1 to 99) until the washings are colorless. Return the paper and precipitate to the original beaker, add 15 ml. of nitric acid, then 15 ml. of sulfuric acid and 5 ml. of perchloric acid. Destroy the organic matter as before, evaporating to strong fumes of sulfuric acid. Cool, dilute with 75 ml. of water, and warm until soluble salts are dissolved. Cool somewhat, add 3 ml. of sulfurous acid (6%), and boil for 5 minutes to expel excess sulfur dioxide. Add 10 drops of nitric acid to the boiling solution and continue the boiling for a minute or two longer. Allow to cool to room temperature.

Filter the solution into a 100-ml. Nessler tube or volumetric flask and adjust the volume to the mark with water. Mix well, and transfer a 10-ml. portion to a photoelectric colorimeter test tube (approximately 12.5 mm. in internal diameter) which has been rinsed with a little of the solution to be read. Read the absorption of the solution, using a filter with mean transmission at 420 millimicrons. (A distilled water blank is satisfactory for the adjustment of the zero point of the colorimeter.) Add 3 drops of hydrogen peroxide (3%), mix well, and again read. The difference between the two readings represents the absorption of the titanium solution. The percentage present is determined from a conversion factor calculated for the instrument by the use of portions of standard titanium solution.

Note. In the absence of molybdenum or vanadium the separation with sodium peroxide may be omitted. In that event, digest the cupferron precipitate with 25 ml. of nitric acid, 15 ml. of sulfuric acid, and 10 ml. of perchloric acid. After evaporation to strong fumes of sulfuric acid, proceed with the solution of soluble salts and treatment with sulfurous acid.

CONCLUSION

Titanium cannot be completely separated from traces of iron, chromium, and nickel by a single precipitation with cupferron. The use of a blank correction for alloys of the same type is not valid since, as it is observed from the initial colorimeter readings in Table I, the blank reading caused by the presence of impurities varies even for separate samples of the same alloy. This variation appears to be governed by the speed of the cupferron addition and the excess added, as well as the degree of air-oxidation of ferrous iron. When the value of the blank for each individual sample is determined and subtracted from the final reading, the results are in excellent agreement with the true values, the average deviation being less than 0.001%.

In the absence of molybdenum over 0.05%, the maximum error introduced by omitting the sodium peroxide separation is 0.001%. Traces of vanadium have a greater effect and should be removed, unless the accuracy desired permits eliminating the additional step. Manganese, silicon, copper, tungsten, zirconium, aluminum, and tin are either removed during the course of analysis or form no interfering color. Columbium interferes, apparently by the formation of a compound with ferric iron upon the addition of hydrogen peroxide. If columbium is present, the procedure is modified to remove the iron by the ammoniacal tartrate–hydrogen sulfide precipitation recommended by Cunningham (*4*); this is best done following the final destruction of organic matter. After removal of the excess hydrogen sulfide, the titanium is reprecipitated with cupferron, digested with the nitric-perchloric-sulfuric acid mixture, and completed as in the regular procedure.

Titanium forms certain compounds which are insoluble in the original solvent acid mixture. These are filtered with the cupferron precipitate and are decomposed upon being fumed with the nitric-perchloric-sulfuric mixture. This decomposition has been found to be complete in all types of steels encountered by

the authors, no titanium being recovered from potassium pyrosulfate fusions of the ignited residues.

Steels of titanium content higher than 0.05% may be analyzed by the method outlined, using proportionately smaller samples. The method may also be used for cast iron, as well as steel of various alloy composition.

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THE opinions expressed are those of the authors and are not to be construed as reflecting the official views of the Navy Department.

Quantitative Spectrographic Method for Analysis of Small Samples of Powders

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A method is described for the quantitative spectrographic analysis of inorganic substances using only 0.1 to 1.0 mg. of powdered sample. When applied to such small samples, the method gives sufficient accuracy for many purposes, and the saving in time is considerable. Calibration curves have been prepared for ten elements and it should be possible to extend this number to include most of the common elements which are excited in the direct current arc. The technique has been found satisfactory for complex rocks, slags, refractories, corrosion products, boiler scale, ash from ignition of organic materials, and various small samples of inorganic nonmetallic powders.

THE development of the technique described was prompted by the need of a rapid method for the quantitative analysis of minute samples of inorganic powders such as corrosion products, nonmetallic inclusions, and ash from organic materials. It has been the authors' experience that the usual quantitative microchemical analysis of such materials is tedious and discouraging. A powder method employing a ball mill for mixing the samples has recently been described by Oshry, Ballard, and Schrenk (*3*). The common desire to secure speed at the expense of accuracy led to the present method, which eliminates several of the time-consuming operations involved in mechanical grinding. In the authors' case the time elapsed between the receipt of the sample in the laboratory and the delivery of the analysis may at most be 2 hours. This allows for preparation of the sample, making any necessary spectrographic adjustments, exposure, processing, photometering, and calculation. A quick analysis of this kind is a very useful preliminary to a complete silicate analysis. The method is an adaptation of the ratio quantitative powder technique of Lewis (*2*), who synthesized powder mixtures until the resulting line intensities of a known mixture matched those of the unknown powder.

The general technique consists of mixing the sample to be analyzed with a large excess of a pure powder mixture which serves as a buffer and internal standard. Weighed proportions of this mixture are then pressed into pellets which are burned in a direct current arc and the spectra prepared. Line intensity ratios between selected spectrum lines of the sample constituents and the internal standard are determined photometrically and converted to weight of constituent by reference to calibration curves.

EQUIPMENT

A Zeiss QU-24 spectrograph and external lens system was used for recording the spectra. This lens system forms an intermediate image of the source so that any part of the arc may be screened off by the choice of a suitable diaphragm. National Carbon Company specially purified graphite electrodes 0.47 cm. ($^3/_{16}$ inch) in diameter were used throughout this work. All photographic plates were Eastman Kodak Company Process plates which were developed in Eastman D-11 solution. A Zeiss spectrum line photometer was used for all line intensity measurements.

PREPARATION OF SAMPLE

Barium nitrate was selected as an internal standard because it is not a common constituent of the materials to be considered, it will not vaporize too rapidly in the arc, it can be obtained readily as a pure salt, and it makes a firmer pellet than other barium salts.

The internal standard-buffer mixture finally chosen was composed of equal parts by weight of barium nitrate and ammonium sulfate. The possibility of the formation of ammonium nitrate in mixing these salts together should not be overlooked. Bearing this in mind, it is not advisable, when preparing pellets, to employ salts of the unknown which have strong reducing properties.

The optimum weight of a standard-buffer was found to be 20 mg. and of sample 1 mg. In certain cases where extremes of concentration were encountered, the sample weight was varied between 0.1 and 5.0 mg. with the regular 20 mg. of standard-buffer.

The sample and standard-buffer were weighed into an agate mortar and ground into a well-mixed fine powder. This was then pressed into a small pellet, using a micropress which forms a cylindrical pill 2 mm. in diameter.

This spectrographic method has been applied to samples weighing 0.1 to 5.0 mg. Although the method has been applied to the determination of ten elements only, there seems to be no reason why this number cannot be expanded to include most of the elements excited by the direct current arc. Work along this line and on other applications is in progress.

EXPOSURE CONDITIONS

A diaphragm, *C*, with a small aperture (0.5 × 10 mm.) was used at the point of intermediate image formation in the external lens system. This aperture was of such size that the optimum

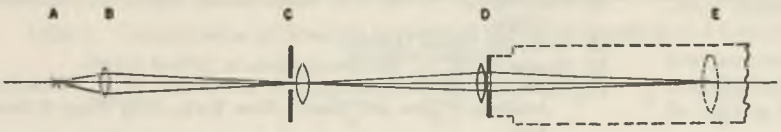

Figure 1. Schematic Diagram of Lens System

exposure was obtained when the sample was burned completely. The lens system employed has been described by Kaiser (*1*), and is shown diagrammatically in Figure 1.

An image of the arc, *A*, is formed on diaphragm *C* by means of an 80-mm. focus quartz lens, *B*. The diaphragm is a rotatable disk containing openings of various heights. By employing the proper opening any portion of the arc column may be used for illumination. The portion of the arc selected for illumination is projected by means of a quartz lens, *D*, of 227-mm. focus onto the collimator lens, *E*. The diaphragm is mounted on a lens of 200-mm. focus which forms an image of lens *B* on the slit of the spectrograph.

The sample pellet was placed in a small crater in the lower positive electrode and arced until it was burned completely. A 220-volt direct current supply was used. With a current of 6 amperes (direct current) between the bare graphite electrodes which were spaced 4 mm. apart, the current rose to about 7 amperes while the pellet was burning, and then dropped back to 6 amperes when the pellet was exhausted. This usually required an exposure of about 2 minutes.

Table I. Wave Length of Spectrum Line Used for Each Element

	Å.		Å.
Ba	2771.4	Cu	2824.4
Sn	2429.5	Mn	2933.1
Si	2435.2	Ni	2943.9
Al	2652.5	Ca	3158.9
Fe	2723.6	Ti	3168.5
Mg	2783.0		

DEVELOPMENT AND PHOTOMETRY OF PLATES

Both the developer and fixer tanks were kept in a water bath at 65° F. A mechanical developer powered by an electric clock motor was used to move the plate back and forth through a 2.5-cm. (1-inch) path in the solution once each minute.

The line intensities were measured on the Zeiss photometer using a galvanometer scale of 1000-ml. divisions. The Leeds & Northrup galvanometer was placed 3 meters from the 1-meter scale. The ratio of the galvanometer deflections for internal standard line and unknown line were plotted against weight of oxide on log-log coordinate paper.

This procedure differs from the more general one of converting the galvanometer deflections to relative intensities by use of a plate calibration curve and plotting the calculated intensity ratios against concentration. The technique employed has been found to save time, since preparation of a plate characteristic curve and conversion of galvanometer deflections to relative intensities are obviated. This saving is at the expense of some precision. The theory and limitations of the method have been adequately covered by Twyman (*4*).

PREPARATION OF WORKING CURVES

Reagent grade oxides of the following elements were used in preparing synthetic standard mixtures for the determination of working curves: silicon, aluminum, magnesium, iron, calcium, titanium, tin, copper, manganese, and nickel (calcium was used as carbonate but calculated as oxide). The first five oxides were varied through the range 0.01 to 1.0 mg., while the last five were considered only in the range 0.01 to 0.1 mg. The composition of each standard mixture was varied widely from the others, so that any effect of one element upon another should show up readily. No such effect was observed.

Each spectrographic plate consisted of several exposures of the five synthetic standards used. A single barium line was chosen for comparison with a line of each of the constituent elements. The wave lengths of the spectrum lines used are given in Table I.

The variations of average line-ratio values for a given concentration of an element were usually less than 20% from plate to plate. When several plates were used to get an average line-ratio value for a large number of determinations, the plot of these averages against concentration yielded working curves with all points falling very near a straight line. The straight lines drawn through the points were calculated by the method of least squares. Several of these curves are illustrated in Figures 2 and 3.

A second set of working curves was prepared by another operator one year after the first set. The old and new curves did not differ by more than 5%, and they coincided in most instances.

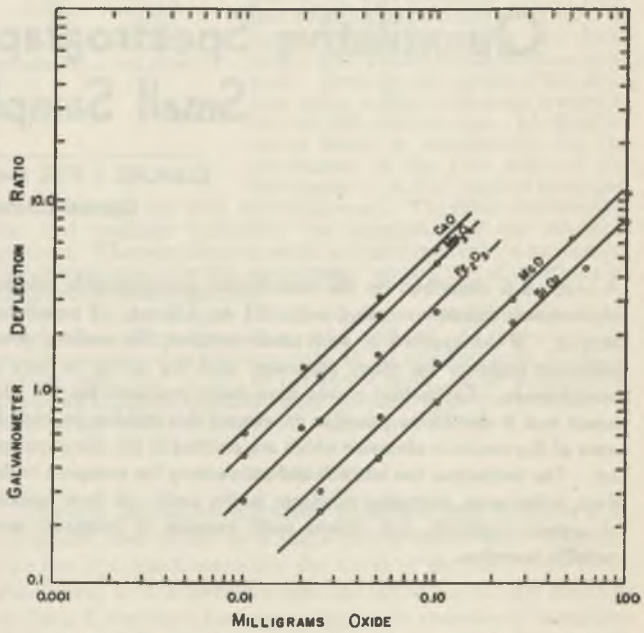

Figure 2

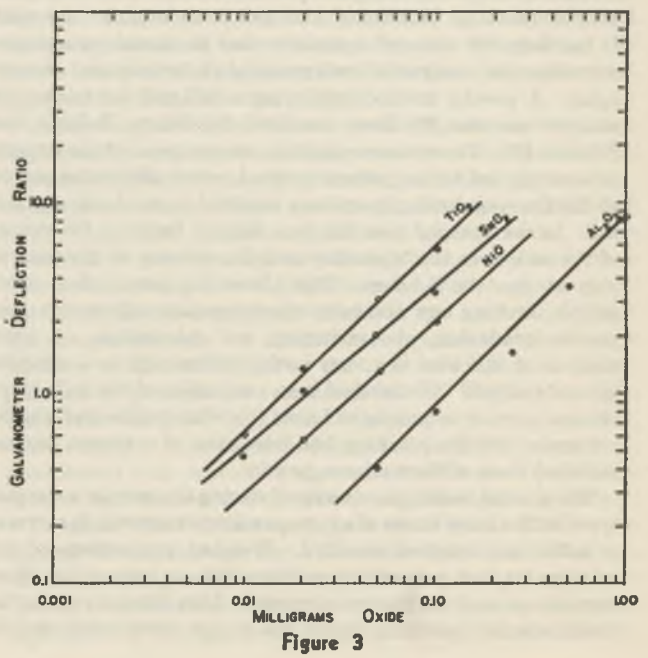

Figure 3

Table II. Application of Method to Analysis of Bureau of Standards Sample[a]

Sample	% SiO_2		% Al_2O_3		% Fe_2O_3		% TiO_2		% MgO		% CaO	
	Chem.	Spec.	Chem.	Spec.	Chem.	Spec.	Chem.	Spec.	Chem.	Spec.	Chem.	Spec.
B.S. 1, argillaceous limestone	18.0	19 19	5.7	6.6 5.3	1.7	1.8 1.6	0.22	<1 <1	1.9	1.9 1.7	38.0	46 43
B.S. 27a, Sibley iron ore	1.0	2.0 2.0	...	...	69.0	48 32	...	...	...	...	...	..
B.S. 69, bauxite	6.3	6.3 6.6	55.0	93 81	5.7	7.8 7.2	3.1	3.9 3.7	...	...	...	..
B.S. 70, feldspar	67.0	58 69	18.0	17 12	0.03	<1	...	...	...	...	...	..
B.S. 76, burnt refractory	55.0	53 59	38.0	37 35	2.4	2.5 2.3	2.2	2.0 1.7	0.58	<1 <1	0.27	..
B.S. 77, burnt refractory	32.0	34 35	59.0	62 59	0.90	1.1 <1	2.9	3.0 2.8	0.50	<1 <1	0.26	..
B.S. 78, burnt refractory	21.0	22 22	70.0	96 69	0.79	1.1 <1	3.4	4.3 4.5	0.51	<1 <1	0.38	..
B.S. 88, dolomite	0.31	<1	0.07	...	0.08	1 <1	...	...	22.0	29 29	30.0	36 37
B.S. 97, flint clay	43.0	48 46	39.0	48 49	0.98	1.0 1.2	2.4	2.9 2.7	0.26	<1	0.10	..
B.S. 98, plastic clay	59.0	64 64	26.0	25 24	2.1	2.3 2.2	1.4	1.5 1.2	0.72	<1 <1	0.21	..
B.S. 99, soda feldspar	69.0	67 60	19.0	20 15	0.07	<1 <1	...	...	0.05	...	0.36	..
B.S. 102, silica brick	94.0	84 59	2.0	2.2 1.4	0.66	<1	0.16	<1 <1	0.21	<1	2.3	1.8 2.3
B.S. 104, burned magnesite	2.5	3.4 2.8	0.84	<2	7.1	7.4 5.7	...	...	86.0	94 61	3.4	2.9 3.4

[a] Each value in "Spec." column represents average of 4 determinations on a single plate, such values from two different plates appearing in table.

Table III. Application of Method to Analysis of Complex Mixtures[a]

Sample	% SiO_2		% Al_2O_3		% Fe_2O_3		% MgO		% TiO_2		% CaO	
	Chem.	Spec.	Chem.	Spec.	Chem.	Spec.	Chem.	Spec.	Chem.	Spec.	Chem.	Spec.
1	37.5	42 41	25.2	21 21	0.5	1 1	7.3	6 7	1.2	1 1	10.9	10 9
2	14.6	15 14	12.8	14 11	23.3	23 20	7.3	6 6	0.8	1 1	10.2	10 8
3	3.3	4 4	19.0	23 26	27.2	26 20	27.8	31 25	1.1	1 1	1.1	.. ..

[a] Each value in "Spec." column is average of two determinations on a single plate, such values from two plates being given.

An average of the two sets of data was used in preparing the curves shown in Figures 2 and 3.

In general the calibration working curves have been reliable to at least 10% for all analysis plates, but when an analysis is being made, a standard mixture is run on each plate to check the working curves. This gives one point on the curve for each element. If this point is found to be in error by more than 10%, a new working curve with the same slope is drawn through this point. Such an adjustment is made only for the plate in question.

If a sample is encountered which contains more than 50% of a single constituent, it is advisable to repeat the determination using only 0.5 or 0.25 mg. of the sample in the pellet. This reduction of the actual weight of the major constituent brings its line density into a lower range which is more suitable for photometry. In the authors' particular case density values between 0.4 and 1.5 were found to yield the best precision. Other types of plates might require utilization of a different range of values.

ANALYSIS OF BUREAU OF STANDARDS SAMPLES

As a test of the reliability and versatility of the method, a group of thirteen National Bureau of Standards samples was analyzed by this technique. Four pellets of each sample were exposed on a plate and two plates were made of each sample. The results of these analyses are given in Table II. The percentage of each oxide in the "Chem." column is taken from the Bureau of Standards certificate for that particular sample. In the "Spec." column, each percentage represents the average from four pellets on a single plate (values from two plates are given). In most cases, the agreement between the true value for an oxide and the value found by this method is very good. In the extreme cases where large errors occur, the constituent is usually present in excess of 50% of the sample.

ANALYSIS OF COMPLEX MIXTURES

Three complex samples were prepared by mixing portions of various Bureau of Standards samples. These mixtures were prepared by another person not familiar with the method, and the composition remained unknown to the operator until after the spectrographic analyses were completed. The "chemical" values for the samples were arrived at from the weight ratios used and the Bureau of Standards certificate for each sample used in the mixture. The mixtures were prepared in such proportions that no single oxide would be present as more than 50% of the whole because the method is most reliable for values below 50%. The agreement between the chemical percentage and the value determined spectrographically was very good for these complex materials (Table III).

Table IV. Analyses of Paper Pulp

	Unwashed Pulp	Washed Pulp
SnO_2	NDA[a]	0.4
SiO_2	11.0	11.0
Al_2O_3	3.7	3.5
Fe_2O_3	1.7	2.2
MgO	17.0	17.0
CuO	1.6	4.3
Mn_3O_4	0.6	0.7
CaO	65.0	61.0
Total	100.6	100.1

[a] No determinable amount.

In Table IV some results are shown which were obtained in the practical application of the method. In this instance it was desirable to know the effects of washing upon the ash content of a particular paper pulp. A quantitative analysis of the respective ashes showed that bronze from the washing machinery was being introduced into the pulp.

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Determination of Fluoride in Water

A Modified Zirconium-Alizarin Method

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A convenient, rapid colorimetric procedure using the zirconium-alizarin indicator acidified with sulfuric acid for the determination of fluoride in water is described. Since this acid indicator is stable indefinitely, it is more useful than other zirconium-alizarin reagents previously reported. The use of sulfuric acid alone in acidifying the zirconium-alizarin reagent makes possible the maximum suppression of the interference of sulfate. Control of the pH of the samples eliminates errors due to the alkalinity of the samples. The fluoride content of waters containing less than 500 parts per million of sulfate and less than 1000 p.p.m. of chloride may be determined within a limit of 0.1 p.p.m. when a 100-ml. sample is used.

VARIOUS methods and modifications using the zirconium-alizarin reagent for the determination of fluoride in water have appeared in the literature (*1*, *2*, *3*, *5*, *6*). The procedure published in 1941 for the determination of fluoride in water, using the zirconium-alizarin indicator and sulfuric acid (*4*), has been revised to make the method more convenient and adaptable for use in water laboratories. The sensitive fluoride range of the indicator has also been increased. The acid indicator used is prepared with sulfuric acid and the zirconium-alizarin reagent. This acid indicator is stable indefinitely, is always ready for use, and is therefore more satisfactory than other previously reported zirconium-alizarin solutions which are not stable. The use of sulfuric acid alone in acidifying the zirconium-alizarin reagent makes possible the maximum suppression of the interference of sulfate. The alkalinity of a sample may interfere with the accurate determination of fluoride by its effect on the pH of the solution. Neutralization of the alkalinity of the samples with nitric acid is employed to prevent errors caused by the alkalinity.

The method reported here embodies a stable indicator solution, the maximum suppression of the interference of sulfate, a sensitive color range, and a more accurate and convenient procedure for the determination of fluoride in water.

REAGENTS

Zirconyl nitrate, 1.84 grams of zirconyl nitrate dihydrate in 250 ml. (filter).

Alizarin red S, 0.37 gram of alizarin monosodium sulfonate in 250 ml.

Sulfuric acid, 2.10 *N* (to 2.12 *N*).

Acid indicator. Add 25 ml. of the zirconyl nitrate solution to 50 to 100 ml. of distilled water and add slowly with constant stirring 25 ml. of the alizarin solution and make up to 500 ml. Mix well and add 500 ml. of 2.1 *N* sulfuric acid. The acid indicator is ready for use in about one hour.

Nitric acid, 0.164 *N* (1 ml. will neutralize 10 mg. of bicarbonate); or use nitric acid that is ten times the strength of the acid used in titrating the alkalinity of the samples.

Sodium fluoride. Stock solution, 0.2210 gram of sodium fluoride in 1 liter. Standard solution, dilute 100 ml. of the stock solution to 1 liter (1 ml. equals 0.01 mg. of fluoride).

PROCEDURE

Transfer 100 ml. of the clear samples to matched Nessler tubes. Make up to 100 ml. in matched Nessler tubes the standards that are needed. Standards may be made up in 0.02-mg. intervals from 0 to 0.16 mg. of fluoride or in 0.01-mg. intervals from 0 to 0.10 mg. of fluoride and in 0.02-mg. intervals from 0.10 to 0.16 mg. of fluoride. Add exactly 10 ml. of the acid indicator to each sample and standard, mix well, and compare in 1 hour or better after the samples and standards have stood overnight. When the alkalinity of the samples exceeds 100 parts per million as calcium carbonate (bicarbonate 122 p.p.m.), neutralize the alkalinity with the 0.164 *N* nitric acid, make up samples and standards to 105 ml., and add exactly 10 ml. of the acid indicator.

Comparisons are conveniently made in a 3-hole colorimeter in which each sample is compared with the two closest standards. For moderately colored waters compensate for the color in the sample as follows: Place the sample to be compared above a Nessler tube containing distilled water, place the standard above a duplicate sample which has been acidified with 5 ml. of 2.1 *N* sulfuric acid, and make up to volume with distilled water. The volume in each tube should be the same.

DISCUSSION OF METHOD

A sensitive color range is obtained for amounts of fluoride ranging from 0.0 to 0.16 mg. which is a range from 0.0 to 1.6 p.p.m. when a 100-ml. sample is used. For waters containing more than about 1.6 p.p.m. of fluoride smaller samples diluted to 100 ml. should be used. It is generally satisfactory to use sulfuric acid that is approximately 2.1 *N* in the preparation of the acid indicator, but to obtain the most satisfactory fluoride range the normality of the acid should be close to 2.1. A sensitive color range is dependent upon the strength of the reagents used in the preparation of the acid indicator.

Although samples and standards can be satisfactorily compared after one hour, comparisons after the samples and standards have stood overnight frequently give a little greater accuracy. This is true for the fluoride methods employing the acid reaction of the zirconium-alizarin indicator because equilibrium conditions are not reached in one hour. The time consumed in adding the acid indicator and in mixing allows slight differences in the stage of the reaction. When comparisons are made after one hour the samples and standards should be of the same temperature and the acid indicator should be added as quickly as possible. The color change is complete for samples and standards that are allowed to stand overnight. In the latter case as much as 2 hours' difference in the time of adding the acid indicator does not affect the determination. This fact can be used to advantage when inspection shows that some of the samples are out of the range of the standards. Smaller samples can be taken, so that when comparisons are made the next day all samples will be within the range of the standards.

By using the sulfuric acid zirconium-alizarin indicator the interference of sulfate is decreased. On the basis of 100-ml. samples the error that may be introduced by sulfate or chloride is as follows: 500 p.p.m. of sulfate are equivalent to about

Table I. Interference of Sulfate, Chloride, and Unneutralized Bicarbonate in Determination of Fluoride in Water

(Using the zirconium-alizarin reagent with 2.1 *N* sulfuric acid)

Sulfate	Fluoride Error (Plus)	Chloride	Fluoride Error (Minus)	Bicarbonate	Fluoride Error (Minus)
		Milligrams per 100 ml.			
20	0.003	40	0.002	10	0.003
30	0.005	60	0.005	20	0.008
40	0.008	80	0.008	30	0.012
50	0.010	100	0.010	40	0.017
60	0.012	200	0.017	50	0.021
100	0.020	...	...	..	...

+0.01 mg. of fluoride; and 1000 p.p.m. of chloride are equivalent to about −0.01 mg. of fluoride. Since the errors introduced by sulfate and chloride are plus and minus, respectively, the effect of the one tends to counteract the effect of the other. However, the effects of interfering ions are not completely additive. On the basis of 100-ml. samples an alkalinity of 200 p.p.m. (bicarbonate 244 p.p.m.) is equivalent to about −0.01 mg. of fluoride. For accurate results it is necessary to neutralize the alkalinity with nitric acid when it exceeds about 100 p.p.m. (bicarbonate 122 p.p.m.). The effect of nitrate in the nitric acid and of nitrate present in natural waters is negligible.

Table I shows the interference of sulfate, chloride, and unneutralized bicarbonate. Bicarbonate may be converted to alkalinity as calcium carbonate by multiplying the bicarbonate by the factor 0.82. The interference of sulfate causes the fluoride measurement to be high and the interference of chloride and unneutralized alkalinity cause the fluoride measurement to be low by the amounts shown in Table I. Neutralization of the alkalinity as prescribed eliminates the error caused by the alkalinity of the samples. Fluoride corrections may be applied instead of neutralizing the alkalinity of the samples. However, neutralization of the alkalinity is recommended.

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Quantitative Estimation of DDT and of DDT Spray or Dust Deposits

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A method for the quantitative estimation of the amount of DDT in a sample containing DDT is based upon the quantitative dehydrohalogenation of DDT under certain conditions. A specific application to the determination of spray or dust deposits is discussed in detail. Data are presented to show both the accuracy and the reproducibility of the method, and possible sources of error are discussed.

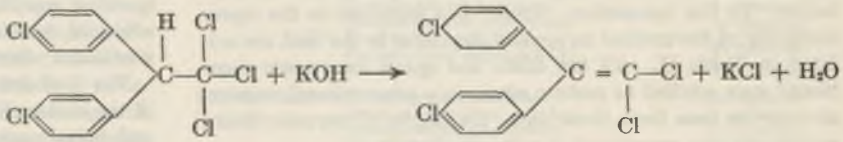
MANY workers have shown DDT [2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane] to be such a promising insecticidal material that the lack of a method of analysis suitable for its quantitative estimation has become a serious hindrance to its further insecticidal evaluation. (The alphabetical symbol derives from the loosely descriptive name, dichlorodiphenyltrichloroethane.) A total halogen determination on a material containing DDT appears impractical because of the probable contamination with extraneous chlorides, such as those found in hard waters, for example. In addition, all organic halogen-containing contaminants, such as those found in technical DDT, would also respond to such a drastic method of analysis.

It has been brought to the attention of the author, and subsequently verified by him, that technical DDT may contain some of the *o,p'* isomer. Theoretically, both isomers should yield one mole of hydrogen chloride under the experimental conditions described herein. Actually, however, slightly more than one mole may be obtained from the purified *o,p'* isomer. This behavior is not without justification, and it will be discussed in detail in a later report.

These considerations led to the development of a more specific method based upon dehydrohalogenation. In 1874, Zeidler (*4*) reported that long boiling of the compound now popularly known as DDT (I) with alcoholic potassium hydroxide resulted in dehydrohalogenation, yielding 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (II). Fischer (*2*) extended this study to several analogs of DDT and reported that most of them readily lost a molecule of hydrochloric acid per molecule of parent compound upon boiling with alcoholic potassium hydroxide.

Recently Brand and Busse-Sundermann (*1*) synthesized and studied DDT and many new analogs. That phase of their work concerned with dehydrohalogenation indicated that 50 ml. of 0.5 *N* alcoholic potassium hydroxide completely dehydrohalogenated 5 grams of DDT within 2 to 3 minutes under reflux.

These studies form the basis for the analytical method herein described. When the sample containing the DDT (I) is treated with excess alcoholic potassium hydroxide, the following transformation is effected:



(I) (II)

Since one, and only one, chloride ion is liberated from each molecule of DDT, the remainder of the method involves determining the quantity of free chloride ion in the products of hydrolysis; this is accomplished by precipitating the chloride ion with excess silver ion, and then determining the excess of the latter by means of a Volhard titration with ferric nitrate as indicator.

PROCEDURE

Strip the treated fruit, leaves, or other objects with benzene, either by shaking them in a Mason jar with the solvent or by washing them individually with a stream of benzene from a wash bottle. The quantity of stripping solvent used is immaterial, for the entire sample will be used in one analysis.

Filter the benzene strip solution through a plug of cotton or of glass wool to remove solid foreign matter, and catch the filtrate in a standard-taper 500-ml. Erlenmeyer flask. If preliminary experiments have shown benzene-soluble inorganic chlorides to be

present in the sample, extract the above filtrate with several portions of water before proceeding to the next step.

Evaporate this solution nearly to dryness on an electric hot plate set for 80° C., and at the same time draw a slow current of air through the flask to hasten evaporation and to prevent the objectionable liberation of benzene vapors into the room. Do not allow the temperature of the residue to exceed 95° C., as DDT may decompose seriously above this temperature.

To the flask add 50 ml. of 1 *N* alcoholic potassium hydroxide, being careful not to wet the ground joint with this alkaline solution.

Fit a standard-taper reflux condenser to the flask and gently reflux the solution on the same hot plate for exactly 30 minutes.

Remove the hot plate, and through the top of the condenser add 50 ml. of distilled water, 50 ml. of 2 *N* nitric acid, and an additional 50 ml. of water, in the order mentioned, to the reaction mixture.

Remove the condenser, and add to the flask approximately 5 ml. of nitrobenzene, followed by exactly 20 ml. of standard 0.1 *N* silver nitrate by means of a calibrated pipet. Stopper the flask with a standard-taper plug and shake it vigorously for a few seconds. This operation coagulates the silver chloride formed, and then coats these particles with a layer of nitrobenzene, so as to prevent their later double decomposition during the titration.

Rinse down the stopper and sides of the flask with water, add 3 ml. of 1 *N* ferric nitrate as indicator, and titrate the excess silver ion with standard 0.1 *N* potassium thiocyanate.

Table I. DDT Recovery Data

Purified DDT Introduced *Mg.*	DDT Found *Mg.*	Recovery *Wt.-%*
1.5	1.5	100.0
9.2	9.3	101.0
100.0	100.3	100.3
262.1	262.8	100.3
311.3	313.1	100.6
514.9	512.2	99.5
624.5	623.2	99.8

ACCURACY AND REPRODUCIBILITY

As outlined above, the method was applied to the quantitative estimations of DDT in various amounts of a strip solution of benzene containing 2 mg. of purified DDT per ml. The results, presented in Table I, show that the variation was confined to 1.5% within the range 2 to 625 mg. of DDT per sample.

Because the method was developed primarily for studies of spray residues, it has been applied assiduously only to this adaptation. In this connection, certain data pertinent to the reproducibility of the method as applied to studies in the field are collated in Table II. All the dusts and spray treatments mentioned were applied to mature citrus in a conventional manner. As may be seen from these data, the degree of reproducibility within any one experiment is very satisfactory.

DISCUSSION

The various quantities of reagents specified in the procedure are those found to be most convenient for the determination of quantities of DDT within the range 2 to 625 mg. per sample. This range may be increased readily by changing the quantity of standard silver nitrate used; a decrease in the range desired will make this a micro process, however, and will thus necessitate the use of a microburet and a weaker standard solution in the titration. Theoretically, the specified amount of alcoholic potassium hydroxide will successfully dehydrohalogenate about 17 grams of DDT per sample.

Benzene was selected as the stripping solvent because at room temperature it will dissolve about 45 weight-% of DDT (*3*), and it will not dissolve appreciably any of the inorganic halides expected to occur in ordinary sprays or dusts containing DDT. For example, inorganic halides in the spray water (hard water) will not be carried over into the strip solution, and thus will have no effect upon the analytical results. Although benzene is an excellent solvent for the hydrocarbon-type oils employed as carriers in most spray mixtures, the oil carried over into the final sample is of no consequence. This fact was demonstrated by adding 4 cc. of kerosene and 4 cc. of a light-medium oil, respectively, to 2 strip samples containing 200 mg. of DDT each; the "recovery" of DDT in both experiments was above 98.5%.

Table II. Reproducibility of Method in Tests on Citrus

Treatment	Sample	Mg of DDT per Sq. Cm. of Surface
3% technical DDT, talc diluent, 1 pound per tree	100 lemon leaves each	4.1 4.3 4.0
6 gals. of kerosene containing 2 pounds of technical DDT per 100 gals. of spray; 1 gal. per tree	100 lemon leaves each	8.5 7.4 8.1
Gesarol AK20[a], 10 pounds per 100 gals. of spray; 1 gal. per tree	100 lemon leaves each	10.4 10.6 10.1
Gesarol SHN20[b], 1.2 gals. per 100 gals. of spray; 1 gal. per tree	100 lemon leaves each	8.2 8.5 8.2
3 gals. of kerosene containing 1 pound of technical DDT per 100 gals. of spray[c]	6 mature grapefruit each	12.6 12.0 12.0
1.75 gals. of light-medium, tank-mix oil containing 0.5 pound of technical DDT per 100 gals. of spray	25 Valencia oranges each	6.0 6.7

[a] A proprietary product of the Geigy Co., Inc., containing 20% technical DDT micronized with pyrophyllite as the diluent.
[b] A proprietary product of the Geigy Co., Inc., containing 20% technical DDT in solution in petroleum oil.
[c] Applied in the laboratory with precision spray equipment.

SOURCES OF ERROR

As mentioned previously, the possibility of errors introduced by inorganic chlorides in the spray materials in general need not be considered. Furthermore, contaminants of organic chlorides in general will not introduce errors unless they are capable of being dehydrohalogenated under the recommended conditions; such compounds will probably not be found in the usual spray or dust mixtures. Technical DDT contains polymers and condensation products of chloral and possibly traces of chlorobenzene; the first two contaminants would not be expected to dehydrohalogenate readily, whereas contamination due to chlorobenzene is of no consequence, for it will not hydrolyze under the specified conditions. Since the reagents may contain traces of chlorides, however, it may be necessary to apply a small correction factor, determined with a blank run, to the results.

The Volhard method is recommended for the determination of the excess silver ion because of its sensitivity. Although the end point may be hard to recognize at first, a little practice enables one to duplicate results very satisfactorily. Overstepping of the end point need cause no concern, for the Volhard end point is reversible.

Only two exact measurements are necessary—that is, one pipetting operation and one titration. All other volumetric measurements are gross, for all are greatly in excess of those theoretically required. Consequently, errors due to faulty readings of volumetric apparatus are reduced to a minimum.

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Walter Ebeling, David Lindgren, and Charles Persing for applying the field treatments mentioned in Table II, and to Leland Brown and Athalie Thomas for valuable assistance in the laboratory.

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Simple Tests for Identification of Sulfonamides

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Simple tests depending on the formation of colored precipitates or solutions with cobalt and copper salts are adapted to the identification of sulfacetimide, sulfadiazine, sulfamerazine, sulfamethazine, sulfanilamide, sulfapyrazine, sulfapyridine, sulfasuxidine, and sulfathiazole. Other alkali-soluble sulfanilamide derivatives would be expected to give similar color reactions with these reagents. Sulfaguanidine and other alkali-insoluble derivatives can be identified by chemical and physical properties which appear elsewhere in the literature.

TESTS for sulfonamides involving reaction of their sodium salts with copper sulfate have appeared in the literature (*1*, *4*, *5*), but, as presented, they do not afford positive differentiation. The purpose of this report is to present a standardized technique for these and supplementary tests, by means of which the most widely employed sulfonamides are easily identified.

Lott and Bergeim (*4*) used this copper sulfate reaction in distinguishing between sulfapyridine and sulfathiazole and characterized the reaction products by means of elemental analysis. They also proposed the quantitative determination of these two drugs, as well as sulfanilamide, by ashing the copper sulfate reaction products and weighing the resulting cupric oxide.

The United States Pharmacopoeia (*5*) and the Council on Pharmacy and Chemistry of the American Medical Association (*1*) make use of the reaction of copper sulfate with the sodium salts of sulfonamides in identifying the latter. Their descriptions of colors for the reaction products, however, are at times misleading and their procedures do not permit reasonably close duplication of results.

PRINCIPLE OF METHOD

On the addition of a solution of copper sulfate or cobalt nitrate to solutions of the alkaline salts of sulfonamides, colored precipitates or solutions, which can be differentiated visually, are produced.

Since most of the copper-sulfonamide precipitates go through series of color changes which at some stages are similar for different sulfonamides, it is advisable to confirm results of these tests, either by timing approximately the periods of color changes of the copper-sulfonamide precipitates or by preforming the cobalt-sulfonamide tests for supplementary information. A further confirmatory test is necessary for sulfanilamide, since it is at times impossible to distinguish between its copper precipitate and a precipitate of copper hydroxide. For this purpose, use is made of the fact that the copper-sulfanilamide precipitate when dissolved in sodium hydroxide and treated with potassium cyanide produces an amber solution, whereas a copper hydroxide precipitate when similarly treated produces a colorless solution.

PROCEDURE

Five milliliters of 0.1 *N* (approximate) sodium hydroxide are measured into a 20 × 150 mm. test tube, and solid unknown material is added in small amounts until there is a definite excess and no more will dissolve.

The solution is filtered through a wet No. 1 Whatman filter paper (7 cm.) and the filtrate collected in a similar test tube. If the filtrate is not clear, it is refiltered through a double thickness of wet filter paper or through a more retentive paper.

Three drops of 15% cupric sulfate pentahydrate or 3 drops of 15% cobaltous nitrate hexahydrate are added to the filtrate. To identify the unknown sulfonamide, the precipitate or color formed is compared with the descriptions given in Tables I and II.

DISCUSSION

Some of the tests described have been used in this laboratory for more than a year and a half, and the color descriptions given have proved adequate to make the tests adaptable for routine use on sulfonamide powders, tablets, and suspensions. Serial color changes are gradual, and time intervals given for such changes are only approximate. Color descriptions should be used only as a guide by individuals in establishing their own impressions of the colors. Colors of precipitates may vary slightly with variations in particle size, but, as a rule, such discrepancies are insignificant. If there is any doubt about the identification of an unknown by this procedure, the test should be repeated with known sulfonamides run simultaneously and under the same conditions. It would be expected that other alkali-soluble sulfanilamide derivatives would give similar specific reactions with the cobalt and copper reagents.

Table I. Cupric Ion–Sulfonamide Color Changes

Sulfonamide	Observed Changes in Order of Appearance	Total Time for Observed Change to Appear
Sulfacetimide	Light yellow-green precipitate	Immediate
	Light yellow-green opalescent solution	5 seconds
	Light yellow-green gelatinous precipitate	10 seconds
	Light greenish-blue sediment Faint greenish-amber supernatant	5–10 minutes
	No change	30 minutes
Sulfadiazine	Yellow precipitate	Immediate
	Yellow-green precipitate	5 seconds
	Green precipitate	15 seconds
	Green-brown precipitate	30 seconds
	Brown precipitate	1 minute
	Purple-brown precipitate	2 minutes
	Brownish-purple precipitate	10 minutes
	Brownish-purple sediment Cloudy supernatant	15 minutes
	No change	30 minutes
Sulfamerazine	Light yellow-green precipitate	Immediate
	Deep green, clear solution	15 seconds
	Gray-green, turbid solution	1 minute
	Gray precipitate	3 minutes
	Gray-brown precipitate	5 minutes
	Blackish-brown precipitate	15 minutes
	Gray-brown sediment Purple-brown supernatant	20 minutes
	No change	30 minutes
Sulfamethazine	Yellow-green precipitate	Immediate
	Dark green, turbid solution	5 seconds
	Orange-brown precipitate	2 minutes
	No change	30 minutes
Sulfanilamide	Greenish-blue precipitate	Immediate
	Blue precipitate	10 seconds
	Blue sediment Colorless supernatant	5 minutes
	No change	30 minutes
Sulfapyrazine	Yellow-green precipitate	Immediate
	Gray-green precipitate	2 seconds
	No change	30 minutes
Sulfapyridine	Yellow precipitate	Immediate
	Light green precipitate	1 second
	Bright green precipitate	10 seconds
	Gray-green precipitate	1 minute
	Light yellow-brown precipitate	5 minutes
	Light brown precipitate	15 minutes
	No change	30 minutes
Sulfasuxidine	Yellow-green precipitate	Immediate
	Gray-green precipitate	5 seconds
	Gray precipitate	30 seconds
	Gray sediment Cloudy supernatant	2 minutes
	Gray sediment Faint amber supernatant	20 minutes
	No change	30 minutes
Sulfathiazole	Greenish-brown precipitate	Immediate
	Brownish-purple precipitate	15 seconds
	Purple precipitate	1.5 minutes
	Dark violet-purple precipitate	5 minutes
	No change	30 minutes

Table II. Cobaltous Ion–Sulfonamide Color Changes

Sulfonamide	Observed Changes in Order of Appearance	Total Time for Observed Change to Appear
Sulfacetimide	Dilution of reagent, no reaction	Immediate
	No change	30 minutes
Sulfadiazine	Deep pink-red solution	Immediate
	Same with gelatinous precipitate	5 seconds
	Clear gelatinous sediment Pink-red supernatant (precipitate may not appear)	5 minutes
	No change	30 minutes
Sulfamerazine	Light pink-white precipitate	Immediate
	Rose, clear solution	10 seconds
	Same with gelatinous precipitate	15 seconds
	Pink gelatinous sediment Rose supernatant	10 minutes
	No change	30 minutes
Sulfamethazine	Pink-white precipitate	Immediate
	Light violet-white precipitate	10 seconds
	No change	30 minutes
Sulfanilamide	Pinkish-blue precipitate	Immediate
	Light blue gelatinous precipitate	30 seconds
	Light blue sediment Colorless supernatant	5 minutes
	No change	30 minutes
Sulfapyrazine	Amber solution	Immediate
	Salmon-pink solution	2 seconds
	Same with gelatinous precipitate	5 seconds
	Pink gelatinous sediment Light amber-pink supernatant	5 minutes
	Bluish-pink sediment Cloudy supernatant	10–20 minutes
	No change	30 minutes
Sulfapyridine	Pinkish-white precipitate	Immediate
	Light violet-pink precipitate	30 seconds
	Pink-white precipitate	3 minutes
	Pink-white sediment Pink supernatant	5 minutes
	No change	30 minutes
Sulfasuxidine	Rose solution	Immediate
	No change	30 minutes
Sulfathiazole	Pinkish-blue precipitate	Immediate
	Violet-blue precipitate	10 seconds
	Violet-blue sediment Lavender supernatant	2 minutes
	No change	30 minutes

Modifications of the tests for special problems have been considered, but they are so obvious and instances so varied that details will not be given here. In some cases, mixtures can be separated by fractional precipitation or solution and the components identified. In such cases, it may be desirable to confirm results of these tests by means of chemical and physical properties of the substances. Calamari *et al.* (*3*) have studied and summarized the chemical and physical properties of some of the most important sulfonamides for purposes of identification.

The blue precipitate formed when cupric sulfate is added to 0.1 *N* sodium hydroxide should be observed as a precaution against mistaking it as a positive copper-sulfanilamide test. This will, of course, be obtained if excess sodium hydroxide is present in the filtrate being examined, so that it is very important to take more unknown material than can be dissolved in the 5 ml. of alkali used for the test. If the unknown happens to be an alkali-insoluble material, such as sulfaguanidine, a questionable precipitate will result; this can be avoided by adding the unknown to the alkali in small amounts and noting whether or not it dissolves. Alkali-insoluble sulfonamides—e.g., sulfaguanidine—can be differentiated and identified by tests such as those of Calamari *et al.* (*3*).

Another test for checking a positive copper-sulfanilamide reaction may be performed as follows: To the reaction mixture containing the precipitate are added 5 ml. of 30% sodium hydroxide followed by 1 ml. of 5% potassium cyanide. The copper-sulfanilamide and copper hydroxide precipitates both dissolve in strong sodium hydroxide to give blue solutions. On the addition of potassium cyanide, such a blue solution resulting from sulfanilamide turns yellow to amber, while one resulting from copper hydroxide is decolorized. Both cobalt and copper reaction products of other sulfonamides react similarly to sulfanilamide with this cyanide test. Arreguine (*2*) reports that sulfanilamide can be detected in the presence of its substitution derivatives by the indophenol reaction, using thymol in ammoniacal medium.

Cobalt-ammonia-sulfonamide complexes have been studied, and although they might be used in supplementing information obtained from tests reported here, they are not so specific and are not considered necessary for inclusion in this paper.

Cerium, chromium, iron (ferric, ferrous, ferricyanide, ferrocyanide), manganese, mercury, nickel, and silver salts were tested in a search for other metals which would give easily differentiated sulfonamide reactions such as those of cobalt and copper, but they were found unsatisfactory. Sodium sulfanilamide with cerium nitrate yields a precipitate of large, clear plates which might be useful for microscopic identification.

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Method for Classification of Petroleum Waxes

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NEW petroleum waxes are constantly appearing on the market, owing to the demand of our Armed Forces and the fact that imported waxes are very scarce or, in some cases, unobtainable. Petroleum waxes have in the past been grouped into two broad classes—crystalline (or paraffin) and amorphous (or microcrystalline). This terminology is now obsolete but is still used to a certain extent by the industry, although it is generally understood that truly amorphous waxes do not exist. The two classes of wax have widely divergent applicabilities, and they have been differentiated by assuming that crystalline waxes are hard and brittle, and that the so-called amorphous waxes are soft and plastic. That this is not necessarily true was indicated by Marcusson and Schlütter (*8*), who showed that ceresine wax (which is hard and dry) is the same as amorphous wax of petroleum. In general, waxes of low molecular weight are more crystalline than those of high molecular weight.

Crystallographic analysis does not differentiate clearly between the two types of waxes. Padgett, Hefley, and Henrikson (*11*) have shown that all forms of waxes occurring in refinery practice, whether crystalline or amorphous, are sufficiently impure to give needle or foliaceous crystals. On the other hand, Buchler and Graves (*1*) have shown that when waxes of low or high melting points, molecular weights, etc., are sufficiently purified, they crystallize in plates. Small amounts of impurities change the plate form to needle form. Finally, Gurwitsch (*5*) states that both natural and artificial petrolatums show a microcrystalline character under the microscope in polarized light.

Possibly this was the reason why the petroleum industry aban-

A simple test is described whereby the crystallinity of petroleum waxes can be measured in relative terms. The test consists in determining the melting point of a wax and measuring the per cent of contraction of a known volume of wax from 10° F. above to 50° F. below its melting point. With these data the degree of crystallinity (called the crystallinity index) of the wax can be calculated. The difference in crystalline structure of waxes and the action of pour-point depressants on waxes are clarified by this test. The procedure could be used as a substitute for or in conjunction with the U. S. Army specifications for cohesion at low and room temperatures.

doned the designation "amorphous" and substituted for it the designation "microcrystalline". This, however, made the confusion even greater, since then there were two types of waxes on the market—crystalline (by imputation, macrocrystalline) and microcrystalline. The waxes should therefore have been distinguished by the size of the crystals. Not only was the crystal size which would make the boundary between the two classes never specified, but the size of crystals would be very difficult to determine in commercial practice. For this reason attempts were made to develop methods for distinguishing between the two types of waxes.

Jones and Blachly (*6*) attempted to differentiate between microcrystalline and macrocrystalline wax by the following test: If a glass rod is dipped into a chilled solution of oil containing a precipitate of microcrystalline wax and is withdrawn, the solution of oil will drain off, leaving only a very thin film of oil adhering to the glass. If the same test is applied to a solution of oil containing a precipitate of macrocrystalline wax, the oil will drain off, leaving crystals of wax adhering to the glass. Jones and Blachly explain this phenomenon by assuming that microcrystalline wax has a greater and macrocrystalline wax has a smaller surface tension than the solution. The authors of this paper have been unable to duplicate these results, but at best this test could be used to differentiate only between the extreme limits of crystallinity. No other reference was found in the available literature to any method whereby micro- and macrocrystalline waxes can be differentiated.

Table I. Per Cent Contraction of Macrocrystalline *vs.* Microcrystalline Waxes

Sample	Melting Point[a] ° F.	Contraction from 200° to 80° F. %
Microcrystalline waxes		
Amber Amorphous A	145	12.3
High viscosity wax B	160	12.5
Tank bottom wax C	188	12.7
Macrocrystalline waxes		
Paraffin wax A	125	13.5
Paraffin wax B	134	15.3
Paraffin wax C	142	16.3

[a] Taken according to A.S.T.M. Designation D127.

PRELIMINARY WORK

Peczalski (*12*) showed that when commercial paraffin (crystalline) wax of 54° C. melting point was kept at 50° C. for 24 hours its specific gravity rose from 0.875 to 0.900. Microscopical examination showed that the crystals had grown larger. His explanation was that certain constituents of the paraffin wax changed its crystalline state with an increase in specific gravity as a consequence. Graefe (*4*) also noted that paraffin wax expands considerably (from 11 to 15%) in melting.

During the course of the authors' work on petroleum waxes it also was noted that on solidification the contraction of microcrystalline waxes appeared to be different from that of macrocrystalline waxes. Accordingly, several commercial samples of these waxes were allowed to cool from 93.33° to 26.67° C. (200° to 80° F.) and the per cent contraction of each sample was noted. The data from Table I show that the macrocrystalline waxes have a higher per cent contraction than the microcrystalline samples, and this test could therefore be used as a rough means for differentiation.

Closer study of the contractions tabulated in Table I shows that whereas an oil has only one type of contraction on cooling, three types of contraction are involved when a wax is cooled from above its melting point to below its melting point:

K_l is the contraction of the liquid wax from 200° F. to its melting point. In this state the wax behaves like an oil and its per cent contraction depends on the molecular weight only. There is no distinction between macro- and microcrystalline waxes.

K_c is the contraction due to the change from liquid to solid state. Its magnitude does not depend on the molecular weight but on the crystallization tendency of the wax. The method suggested in this paper indicates that the K_c of an amorphous wax should be zero. The greater the crystallinity of the wax the greater should be its K_c.

K_s is the contraction of the solid wax from its melting point to 80° F. The magnitude of this contraction probably depends primarily on the molecular weight of the wax and does not measure its crystallinity.

Table II. Constancy of K_l for Different Waxes

Samples	Density at 200° F.	Saybolt Viscosity at 210° F.	Melting Point, ° F.	K_l per ° F.
Tank bottom wax C	0.8078	77	188	0.035
Amorphous wax B	0.8074	118	160	0.037
Amorphous wax D	0.7883	62	165	0.037
Paraffin wax D	0.7717	50	163	0.039
Paraffin wax B	0.7558	38	135	0.043

Hence the total contraction, K_t, is equal to $K_l + K_c + K_s$; but macro- and microcrystalline waxes could be most sharply differentiated by measuring K_c only. This could be done in two ways: (1) The contraction of the wax sample should be determined at its melting point. Unfortunately, petroleum wax (especially of the microcrystalline type) does not have a sharp melting point, but rather a melting point range, the magnitude of which depends on the molecular weight range and chemical composition of the individual components of the wax. (2) The K_l and K_s could be determined for each individual wax and subtracted from K_t, thus yielding the K_c by difference.

DETERMINATION OF K_l AND K_s

Samples of commercial waxes (100 ml.) of widely divergent properties were allowed to cool at the rate of 10° F. per hour in a thermostatically controlled bath, and the average per cent contraction per 0.556° C. (° F.) was noted for the temperatures ranging from 93.33° C. (200° F.) to about 2.7° C. (5° F.) above the melting point of each sample (Table II). The results show that the value 0.04 can be used for the calculation of K_l for all waxes with probably a maximum error of ±0.005. This would not appreciably affect the accuracy of the method, and would greatly simplify the test.

Although the value of the K_l of the individual waxes remained more or less constant for all the temperature ranges from 200° F. to the melting point, it was noted that the K_s had no such constant value. All the wax samples yielded large values for K_s im-

Table III. Values for K_s of a Typical Macro- and Microcrystalline Wax

Paraffin Wax D, 163° F. M.P.		Amorphous Wax B, 160° F. M.P.	
Temp. range, ° F.	K_s/° F.	Temp. range, ° F.	K_s/° F.
160–150	0.530	157–147	0.230
150–140	0.333	147–137	0.188
140–130	0.144	137–127	0.170
130–120	0.078	127–117	0.130
120– 80	0.040	117– 80	0.077
Av.	0.155		0.130

Table IV. Crystallinity Index

Sample	Melting Point, ° F.	Viscosity at 210° F., Seconds	Lovibond Color, 0.5-Inch Cell	Penetration	Total Contraction, %	Crystallinity Index
True Microcrystalline Waxes						
Amorphous B	160	118	6Y1/4R	28	8.9	65
Black Amorphous G	180	96	Black	18	9.2	68
Microwax A	160	90	Dark	32	9.3	69
Amber Amorphous K	148	50	35Y22R	50	9.3	69
Amber Amorphous H	181	69	35Y45R	22	9.4	70
Yellow Amorphous I	160	49	35Y2R	37	9.4	70
Green Amorphous E	181	90	Dark	19	9.5	71
Microwax B	140	84	Dark	40	9.8	74
Tank bottom wax C	188	77	35Y16R	28	10.0	76
Ceresine	187	59	35Y4 1/2R	8	10.4	80
Semicrystalline Waxes						
Lemon wax O	157	49	35Y1 1/4R	15	11.4	90
Wax D	165	62	10Y1/2R	19	11.7	93
Wax P	162	62	35Y6R	25	12.5	101
Wax M	166	64	35Y5R	25	12.5	101
White ozokerite	150	41	1/2Y	18	12.7	103
Macrocrystalline Waxes						
Scale wax F	132	39	2Y	21	13.3	109
Paraffin wax A	125	39	White	18	13.7	113
Imported paraffin I	140	40	White	11	13.7	113
Paraffin wax D	163	50	1Y	18	13.9	115
Paraffin wax H	124	38	White	17	14.0	116
Paraffin wax B	134	39	White	15	14.1	117
Imported paraffin G	148	41	White	13	14.1	117

mediately below their melting points and these values decreased as the temperature was lowered. This is shown in Table III and Figure 1, wherein samples of macrocrystalline and microcrystalline wax of approximately the same melting point were studied. The large contraction immediately below the melting point of the wax is due not simply to thermal contraction but also to the change in structure of the solid wax with a resultant contraction. This phenomenon has also been observed by Carpenter (*2*), Müller (*9*), Yannaquis (*14*), Scott-Harley (*15*), and Lord (*7*). Hence, it would be incorrect to subtract from K_t the average values for K_s as listed in Table III, since these values include also the contraction due to crystal structure change. This part of the contraction should, of course, be included in K_c but cannot readily be obtained experimentally.

However, it can be argued that an ideally amorphous wax would contract in the solid state as if it were a liquid—it would give a constant K_s which would be identical with its K_l. Thus the contraction of the waxes should be studied through the same temperature range with respect to their melting points and not through the same absolute temperature range; and the temperature range should be broad enough to ensure that all contraction due to crystal structure change has ceased.

Hence in the subsequent experiments the contraction of all of the waxes was determined from 10° F. above to 50° F. below their melting points.

DEFINITION OF CRYSTALLINITY INDEX

Let us designate the degree of crystallinity of a wax by the term "crystallinity index", which is taken as 10 K_c, where:

$$K_c = K_t - (K_l/^\circ \text{F.} \times 10 + K_s/^\circ \text{F.} \times 50) \quad (1)$$

or since $K_s/^\circ$ F. can be taken as equal to $K_l/^\circ$ F. if the procedure as outlined above is followed,

$$K_c = K_t - K_l/^\circ \text{F.} \times 60 \quad (2)$$

Since $K_l/^\circ$ F. is taken as 0.04 for all waxes,

$$K_c = K_t - 2.4 \quad (3)$$

$$C.I. = 10\ (K_t - 2.4) \quad (4)$$

where $C.I.$ is the crystallinity index, K_t is the total per cent contraction of the wax from 10° F. above its melting point to 50° F. below its melting point, K_s is that portion of the per cent contraction of solid wax which is due to thermal change and not due to crystal structure change, and K_l is the per cent contraction of the wax in the liquid state.

PROCEDURE

Pour exactly 100 ml. of the wax (whose melting point has been determined according to A.S.T.M. Designation D127) heated to 10° F. above its melting point into a 100-ml. mixing cylinder which has been heated to the same temperature. Allow it to cool for 2 hours protected from drafts and then for 2 hours in a water bath kept at 50° F. below the melting point of the wax. As the wax cools it will form a cavity running down the center of the cylinder, often covered by a thin layer of wax. Pierce the wax layer at the center with a sharp instrument, such as a pointed glass rod, so that a hole about 2 to 3 mm. in diameter is formed. Add (from a buret) a 50% aqueous solution of glycerol to the 100-ml. mark. Apply a slight vacuum to liberate any trapped air and refill to the 100-ml. mark. The amount of liquid added is the total per cent contraction.

Other types of containers than measuring cylinders did not yield reproducible results. For example, a 100-ml. volumetric flask and a 100-ml. Cassia flask did not yield reproducible results, because of the impossibility of removing all the entrained air. Water is not suitable as the titrating agent, owing to its low wetting power. Water alone gives a meniscus which is hard to read and does not readily penetrate the narrow crevices formed, especially those crevices which are formed when the wax cake shrinks away from the sides of the vessel, as is the case with many paraffin waxes. The low surface tension of aqueous glycerol overcomes this obstacle. The results are reproducible within 0.5 cc.

EXPERIMENTAL DATA

In Table IV are listed the crystallinity indexes of various commercial wax samples arranged in ascending degree of crystallinity. The viscosities are all Saybolt Universal; the colors were taken with a Lovibond tintometer, using standard red and yellow glasses; and the penetration was taken according to the A.S.T.M. penetration of bituminous materials test, Designation D5–25. There is unfortunately no reliable test for the determination of oil in waxes (especially of the microcrystalline type) but waxes with a penetration less than 20 could be assumed to contain less than 1% oil; and with the possible exception of two samples (Amber Amorphous K and Microwax B) none of the waxes probably contained more than 5% oil.

DISCUSSION OF RESULTS

It is to be noted from Table IV that there is no sharp line of demarkation between microcrystalline and macrocrystalline waxes. The microcrystalline waxes are products of high viscosity and dark colors indicating the presence of impurities. They are mainly residual products, with the possible exception of samples K, H, and I which may be overhead fractions. The semicrystalline waxes are known to be overhead fractions of relatively high viscosity with the exception of the sample of white ozokerite, which is supposedly a residual product. The relatively high crystallinity of the white ozokerite could be explained by the fact that it might be adulterated with paraffin wax, as was the case with much of our imported ozokerites and ceresines. The macrocrystalline samples are all paraffin waxes and are of course the most crystalline. The scale wax sample, F, being only partially refined, still contains a small amount of impurities and consequently is the least crystalline of this group. This test should, of course, be run on relatively oil-free waxes, although the presence of a small amount of oil does not greatly interfere with its results (Table V).

AMORPHIZING AGENTS

Generally speaking, the more viscous, dark waxes are microcrystalline and the low-viscosity, light-colored waxes are macro-

Table V. Effect of Amorphizing Agents on Crystallization of Wax

Sample	Melting Point, ° F.	Total Contraction, %	Crystallinity Index
Paraffin wax B plus:	134	14.1	117
5% fully refined white mineral oil	132	13.6	112
2% pour-point depressant A	133	11.1	87
5% pour-point depressant A	131	8.9	65
5% pour-point depressant B	133	9.5	71
5% Pa. residue of 250 viscosity at 210° F.	132	10.8	84

crystalline. This agrees very well with the theories of many investigators who have indicated that amorphous waxes are caused by the adsorption of certain impurities which could be called amorphizing agents (*1*, *5*, *6*, *11*). Hence, addition of amorphizing agents to a macrocrystalline (commercial paraffin) wax should make the latter more or less microcrystalline, and this test should detect the conversion if the test is at all dependable. For example, Davis and Zimmer (*3*) have shown that the addition of small amounts of Paraflow (a pour-point depressant) to a wax-bearing oil inhibits greatly the growth of wax crystals.

That the crystallinity index is lowered by the addition of a pour-point depressant or a viscous dark residual oil to a wax of a high crystallinity index is shown in Table V. Five per cent of fully refined white mineral oil (70 viscosity at 100° F.; 0 Iodine No.) was also added to one sample of crystalline wax to prove that the reduction in crystallinity index is not due to the simple addition of oil.

Conversely, removal of these amorphizing agents, naturally occurring or artificial, tends to increase the crystallinity of a wax, and this test should be able to detect the change. Accordingly, a sample of crude Pennsylvania microcrystalline wax was highly refined and its crystallinity index increased from 67 to 82 (Table VI).

Table VI. Effect of Removal of Amorphizing Agents

Sample	Melting Point, ° F.	Color	Crystallinity Index
Crude Pa. microcrystalline wax	131	Black	67
Refined Pa. microcrystalline wax	130	White	82

APPLICATIONS OF CRYSTALLINITY INDEX TEST

There is a great demand by our Armed Forces for microcrystalline waxes which must pass U. S. Army Tentative Specification AXS-1015. One specification (*10*) requires that the wax show good cohesion at low temperatures (no flaking and preferably no cracking). Another specifies that it should not show cracking at room temperature. These tests are not easy to perform and the results are difficult to interpret; consequently both the Army and the petroleum industry are having considerable trouble setting up specifications to take care of this property. The data show (Table VII) that the low-temperature cohesion runs roughly parallel with the crystallinity of the wax. Hence the crystallinity index could be used as a substitute for or in conjunction with the cohesion tests and specifications could be set up in mathematical terms which could be readily determined in the laboratories. Thus the cohesion of waxes is related to their crystallinity indexes in some such manner:

Crystallinity index > 100 flaking
= 100–110 cracking
= 90–100 slight cracking
< 90 acceptable

Table VII also indicates that the crystallinity index can be used to detect adulteration of microcrystalline with commercial paraffin (macrocrystalline) wax, since a mixture of both tends to have an index higher than its arithmetical average.

GENERAL DISCUSSION

The proposed test for determining the crystallinity index of petroleum waxes is based partly on the assumption that the extent of thermal contraction of an ideally amorphous wax in its solid state is the same as its thermal contraction in the liquid state, and any deviation therefrom is due to the crystallinity of the wax. However, the test appears to grade waxes of known origin in their correct sequence with respect to their degree of crystallinity. That macrocrystalline waxes should show a greater contraction than microcrystalline waxes when passing from the liquid to solid state is to be expected, since the intermolecular distances within a crystal are probably much smaller than distances between discrete particles.

Confirmation that this test is reasonably correct can be seen in the fact that it checks with the well-known theory that the so-called amorphous waxes are not amorphous per se, but that the formation of large crystals is inhibited by the presence of certain

Table VII. Crystallinity Index *vs.* Cohesion at Low Temperatures

Sample	Crystallinity Index	Low-Temperature Cohesion
Pa. microcrystalline wax	80	Acceptable
75% Pa. microcrystalline wax + 25% paraffin wax	94	Acceptable
50% Pa. microcrystalline wax + 50% paraffin wax	108	Slight cracking
25% Pa. microcrystalline wax + 75% paraffin wax	115	Much cracking
100% paraffin wax	117	Flaking
Amorphous wax B	65	Acceptable
Lemon wax O	90	Slight cracking

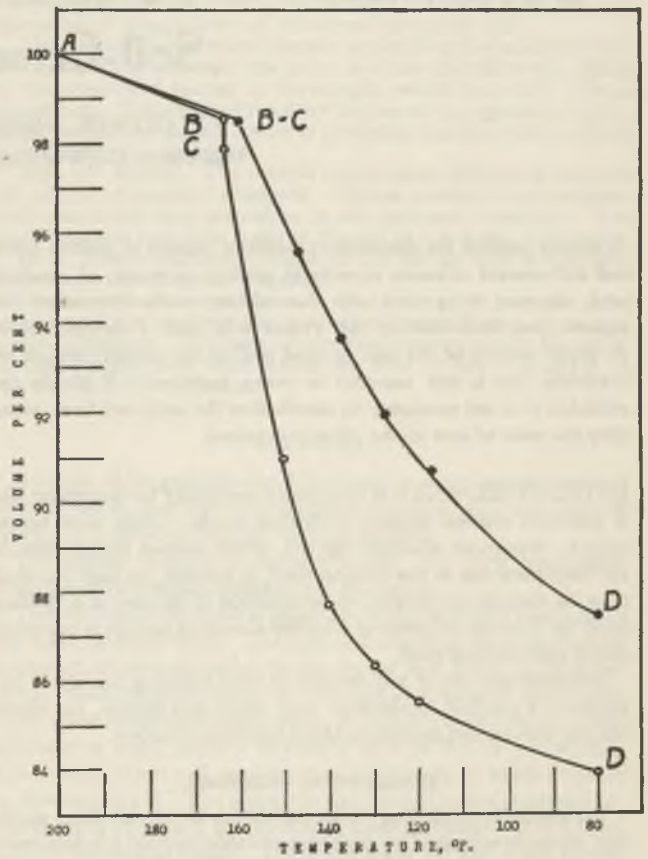

Figure 1. Volume-Temperature Curves of Waxes

○. Paraffin wax D, 163° F. m.p.
●. Amorphous wax B, 160° F. m.p.
AB. Liquid wax contraction, K_l
BC. Contraction due to solidification, K_s
CD. Solid wax contraction, K_s

types of impurities (amorphizing agents). It also confirms the experience of the paraffin wax industry. Zaloziecki (*15*), who first studied the crystallinity of waxes, was of the opinion that the wax in crude oil was always in the amorphous state and he designated it as "protoparaffin". This was converted to the crystalline wax or pyroparaffin by heat during distillation of the crude oil. Undoubtedly Zaloziecki never worked with pure wax and his products always contained amorphizing substances. In the manufacture of paraffin waxes it is necessary to eliminate amorphizing substances and obtain the wax in the crystalline state; otherwise it could not be successfully pressed and sweated. At first the petroleum industry followed Zaloziecki's theory, and by moderate cracking converted the "protoparaffins" to "pyroparaffins". This was later found to be unnecessary when better fractionation of the wax distillate was accomplished. Instead of being destroyed by cracking, the amorphizing substances were separated from the wax by better fractionation. Finally, the results listed in Tables V and VI, correlating the action of amorphizing agents on the crystallinity index, are further confirmation of the validity of this test.

Assuming that this test is valid, it is apparent that the so-called amorphous waxes are microcrystalline in nature and that the only difference between the crystalline and "amorphous" waxes is a matter of crystal size (disregarding the types of crystals).

According to Davis and Zimmer (*3*) and as the crystallinity index of Paraflowed wax shows, the action of pour-point depressants may be partially explained by the fact that they inhibit the growth of wax crystals. A wax-oil mixture, which upon cooling forms smaller crystals due to the presence of pour-point depressants, solidifies at lower temperatures. This is probably because small crystals cannot form an interlocking structure as readily as large crystals.

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Determination of Portland Cement in Drilling Muds and Soil-Cement Mixtures

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A simple method for determining portland cement in drilling muds and soil-cement mixtures consists of adding an excess of standard acid, allowing it to react with the calcium oxide content of the cement, and back-titrating the excess acid with a standard base. A blank sample of the soil or mud and of the cement should be available, but is not essential in many instances. If blanks are available it is not necessary to standardize the acid and base, since only the ratio of one to the other is required.

IN OIL-FIELD work it is frequently necessary to determine the portland cement content of drilling muds. Often it is necessary to determine whether cement, which causes flocculation of the clay particles in the drilling mud, is present, so that the mud may be chemically treated to recondition it for use as a drilling fluid, or in order to discover whether a cement sample is contaminated with drilling mud.

Recently the use of soil-cement in road building has made imperative a method, preferably very rapid and simple, for determining the cement content in these soil-cement mixes.

EXPERIMENTAL PROCEDURE

Six methods, including three variations of one of these methods, were investigated to develop a reliable method for determining the cement contamination of drilling muds or soils. In all cases, except for neat cement slurry blanks, weighed amounts of cement were added to weighed samples of drilling mud. The solid material of the drilling mud was then determined upon a separate sample, being dried at 110° C. to constant weight, which enabled the authors to obtain the per cent cement based on the total solid material present.

Sulfate Determination. This is the method proposed by various cement manufacturers. A sample of the soil-cement, or cement-contaminated mud, is leached several times with water. A sulfate determination is then made on the filtrate using barium sulfate. This is based on the fact that about 2% calcium sulfate is added as a retarder in the manufacture of cement. Since the calcium sulfate content of cements is so low and variable, blanks on both soil and cement are required. Great accuracy is called for, since the factor for converting sulfate to cement is very large.

The disadvantages of this method are the slow filtration, often over 24 hours for one sample, and the great inaccuracy, which is shown in Table I.

A.S.T.M. Silica Determination (*1*). The sample is treated with hydrochloric acid and filtered. The residue is treated with sodium hydroxide and filtered, and a silica determination is made on the combined filtrates.

Cements usually contain about 21% silica. Blanks on the soil or mud used are essential and cement blanks are desirable. However, in many cases, especially cement cut muds, the value 21% is sufficiently accurate for the cement blank. The conversion factor is relatively small and in cases where great accuracy is not required, the cement blank can very well be omitted.

The greatest criticism of this method is the time consumed in filtration, usually over 24 hours for each sample. The accuracy of the method is not very great (Table I).

Calcium Determination. The sample is treated as in the silica determination and a calcium determination is made upon the silica filtrate.

Cements are usually composed of about 65% calcium oxide. This gives a very good factor for conversion to cement and more often than not a cement blank will not be required. A soil or mud

blank is usually required; however, on rough work even this blank may become unnecessary.

We are still confronted with the slow filtration found in the A.S.T.M. method for silica, plus the additional separations required. The accuracy gained is not appreciably greater than that of the A.S.T.M. method for silica.

Table I. Determination of Portland Cement

Test No.	Cement Added[a], %	Cement Found[a], %	Difference
	Sulfate Determination		
1	23.0	36.0	+13.0
2	48.0	53.0	+ 5.0
3	65.0	96.0	+31.0
	A.S.T.M. Method for Determining Silica		
1	3.4	8.0	+ 4.6
2	18.0	12.0	− 6.0
3	23.0	24.0	+ 1.0
6	42.0	18.0	−24.0
7	48.0	40.0	− 8.0
8	65.0	58.0	− 7.0
	Calcium Determination		
1	3.4	14.0	+10.6
2	18.0	13.0	− 5.0
3	23.0	23.0	± 0.0
6	42.0	37.0	− 5.0
7	48.0	42.0	− 6.0
8	65.0	56.0	− 9.0
	Determination of Silica Using Carbonate Fusion		
6	3.4	4.0	+ 0.6
7	18.0	18.0	± 0.0
8	42.0	44.0	+ 2.0
	Determination of Silica Using Perchloric Acid		
6	3.4	2.0	− 1.4
7	18.0	14.9	− 3.1
8	42.0	36.3	− 5.7
	Volumetric Method		
	Bromocresol Green–Methyl Red Indicator		
6	3.4	4.0	− 0.6
7	18.0	18.0	± 0.0
8	42.0	43.0	+ 1.0
1a	0.65	0.86	+ 0.21
1b	2.56	2.33	− 0.58
1c	6.2	6.8	+ 0.6
2a	0.65	0.59	− 0.06
2b	1.30	1.76	+ 0.46
2c	6.2	7.0	+ 0.8
2d	24.8	23.5	− 1.3
3a	0.65	0.54	− 0.11
3b	1.30	2.17	+ 0.87
3c	2.56	3.79	+ 1.23
3d	6.2	7.5	+ 1.3
9a	1.31	1.70	+ 0.39
	Phenolphthalein Indicator		
9b	1.96	2.25	+ 0.29
9c	3.85	3.94	+ 0.09
9d	7.37	7.11	− 0.26
9e	11.47	11.25	− 0.22
9f	13.17	12.96	− 0.21
	pH Meter Variation		
6a	3.40	4.07	+ 0.67
6b	8.1	8.6	+ 0.5
6c	15.0	15.6	+ 0.6
6d	30.7	29.8	− 0.9
7a	0.88	1.45	+ 0.67
7b	8.1	8.7	+ 0.6
7c	15.0	16.2	+ 1.2
7d	30.7	28.8	− 1.9
	High pH Method		
8a	0.61	0.66	+ 0.05
8b	0.96	1.05	+ 0.09
8c	1.29	1.12	− 0.17
8d	1.80	1.64	− 0.16
8e	1.86	1.86	± 0.0
8f	2.37	2.26	− 0.11
8g	2.94	2.71	− 0.23
8h	3.40	3.14	− 0.26
8i	3.79	3.85	+ 0.06
8j	4.53	4.57	+ 0.14
8k	5.95	6.05	+ 0.10
8l	6.26	6.40	+ 0.14
8m	7.12	7.08	− 0.04
8n	8.12	8.28	+ 0.16
8o	8.74	8.59	− 0.15
8p	9.47	9.26	− 0.21
8q	9.65	9.60	− 0.05
8r	10.15	10.25	+ 0.10
8s	10.71	11.00	+ 0.29
8t	12.36	12.30	− 0.06
8u	12.48	12.41	− 0.07

[a] $\frac{\text{Weight of cement} \times 100}{\text{weight of cement + weight of dry solids}}$ = per cent cement

Silica from Carbonate Fusion. A sample of the mixture is fused with sodium carbonate, dissolved in water, and acidified with hydrochloric acid, and the silica is determined.

Blanks of soil are essential to this method. Cement blanks are very desirable but in rough work may sometimes be omitted.

This method is inherently slow, since a fusion and a long slow evaporation to dryness are required. Where time is of no consequence, this method appears to be very good.

Silica Using Perchloric Acid. The sample mixture is treated with dilute perchloric acid, taken to fumes, diluted, and filtered to obtain the silica.

Soil blanks are essential and cement blanks desirable as in the other methods using silica for the cement determination. The remarks made above apply to this method. No great accuracy was found with this method; however, it is relatively fast.

Volumetric Method. Three variations were used in this method. Basically all are the same and the variations were used as checks against each other, so that available equipment could be utilized to the best advantage.

Indicator Variation. An excess of standard 0.5 *N* hydrochloric acid was added to 1.00 gram of the dried mixture. This was brought to a boil, allowed to cool, reboiled, and diluted to approximately 150 ml. A few drops of mixed bromocresol green–methyl red indicator were added, and the excess hydrochloric acid was titrated to a purple or green end point with 0.2 *N* sodium hydroxide. This is based on the following reaction:

$$2H^+ + CaX_2 \longrightarrow Ca^{++} + H_2X_2$$

where *X* is used to represent one equivalent of the slightly soluble acids, of which cement is commonly supposed to consist, or an equivalent of oxygen.

In one instance, phenolphthalein was used as the indicator to find out whether the indicator was critical (see Table I).

pH Meter Variation. The sample was treated exactly as in the indicator variation, except that the back-titration was made with 1.0 *N* hydrochloric acid, using a Beckman pH meter to obtain the end point which was found to be pH 4.5. This end point was obtained by plotting pH *vs.* ml. of sodium hydroxide used.

This method is somewhat simpler to use than the indicator variation, since the indicator end point is often difficult to see, owing to the suspended matter in the sample, which is usually colored somewhat. This method requires the use of an expensive instrument and unless the pH meter is available, the indicator method is probably to be preferred.

High pH Method. The sample was covered with water and the pH of the suspension obtained. Excess standard hydrochloric acid was added and treated as in the indicator variation. The back-titration was then made to the pH of the water suspension.

In all three variations a blank on the soil or mud is essential and one on the cement is desirable.

Several different cements were used, using the various volumetric methods to check the method against different types and makes of cement. This was not deemed necessary with the other methods, since they were either too slow or too inaccurate to merit further investigation.

DISCUSSION

Because of long-drawn-out filtrations, in most cases requiring over 24 hours, the first four methods are slow, and with the exception of the fusion method, very inaccurate.

The sulfate method is relatively fast, but very inaccurate, since the sulfate is such a small part of cement.

The volumetric method is faster and more accurate than any of the other methods tried. The data indicate that phenolphthalein indicator, or back-titration to the initial end point, is more accurate than back-titration to the mixed indicator end point. With cement-contaminated muds, it makes no difference which indicator is used, since a deviation of 1 or 2% is permissible. Soil-cement mixes will require phenolphthalein or back-titration to the original pH. The cement in soil-cement mixes is usually between 4 and 16% and, in this range, these methods will be within 0.2% of the actual values. Using the lower pH end point the results will be within 0.6% of the actual value. The accuracy required will determine the best method. The mixed indicator end point is easier to see than the phenolphthalein end point. With the pH meter, the high pH method should be used.

Table II. Determination of Portland Cement

Cement No.	% Cement per Ml. of 0.5 *N* HCl: Hydrated cement	Dry cement
1	2.86	3.70
2	2.94	3.84
3	2.71	3.52
4	2.55	3.32
5	2.42	3.15

SUPPLEMENTAL INVESTIGATION

Inasmuch as both soil-cement mixes and cement-contaminated muds would probably contain hydrated cement, it was thought necessary to check the volumetric method using five hydrated coments. Neat cement slurries were allowed to set with an excess of water present. A sample was then taken and the per cent cement determined using the pH meter variation. Using a 1.000-gram sample the results reported in Table II were obtained.

This is as was expected, since hydrated cement has taken on water and contains less than 65% calcium oxide. Hence, hydrated samples must be checked against hydrated cement blanks when necessary.

CONCLUSION

Many methods have been suggested and tried with varying success. The authors have evolved a method which is simple and considerably faster than any method described in the literature. While this method is not extremely accurate, and blanks of the soil and the cement used are desirable, it is accurate enough for most practical purposes.

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Glass-Surfaced Magnetic Pump

For Circulating Liquids in a High-Vacuum System

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IN CONNECTION with the circulation of fats and oils in a large cyclic falling-film molecular still (*1*), a pump was designed to meet the following principal requirements: to operate in a system whose pressure may be as low as 10^{-5} to 10^{-6} mm. of mercury, to avoid contamination of the cycled fluid, to have no dead spaces that keep portions of the liquid from circulating, to pump automatically several hundred milliliters of liquid per minute, and to drain by gravity and be easily cleaned.

Several pumps with various types of valves have been described in the literature, but none was found that would meet all requirements satisfactorily. A pump having a hollow plunger and ball-and-tail valves was used by Quackenbush and Steenbock (*4*) on a new molecular still, but it had insufficient pumping capacity to meet requirements. A pump of earlier model having a hollow plunger and ball valves has been described by Hickman (*2*) and by Rosenberger (*5*). Other pumps, more complex in design, are difficult to operate in a high vacuum and may contaminate the cycled liquids.

A complete apparatus designed to fill the above requirements is illustrated in Figure 1. In general, it may be used to pump any liquid noncorrosive to glass against a considerable head.

The all-glass-surfaced pump, *B*, is a single-acting type employing electromagnets to operate a solid piston. The valves are very sensitive and permit a volume efficiency for the pump of 95% based on a measured delivery of 17 ml. per stroke at a rate of 255 ml. per minute. The holdup in the entire pumping unit is very small, since the liquid in the pump after each stroke is relatively negligible. The pump is automatically operated, and its speed is controlled through an eddy-current flasher motor, *C* (flasher motor 3-6172, Sangamo Electric Company, Springfield, Ill.). Hand switches, shown below *C*, may also be used to operate the pump.

By a simple adjustment of the flasher motor and proper spacing of the solenoids, the length of a stroke of the plunger and the ratio of the time of its upward to its downward stroke may be set for the highest pumping efficiency.

The 4-tube rectifier, *A*, shown in Figure 1 is used as a direct-current power supply for the solenoids.

The construction of the pump is shown in detail in Figure 2.

The plunger, *P*, was made from a glass tube which was ground together with the glass cylinder of the pump in a manner that permitted free and smooth movement of the one inside the other. A mild steel rod, *J*, was sealed inside the plunger shell together with a small piece of asbestos at each end to hold the rod firmly in place. The hook, *H*, on top of the plunger may be used to remove it from the pump through the large ground-glass joint, *B*. The plunger is approximately 2 cm. in diameter, 11.5 cm. long, exclusive of the hook, and weighs 160 grams complete.

A depression, *F*, at the bottom of the cylinder of the pump, holds a woven fiber-glass cord, *G*, to cushion the fall of the piston when the pump is empty.

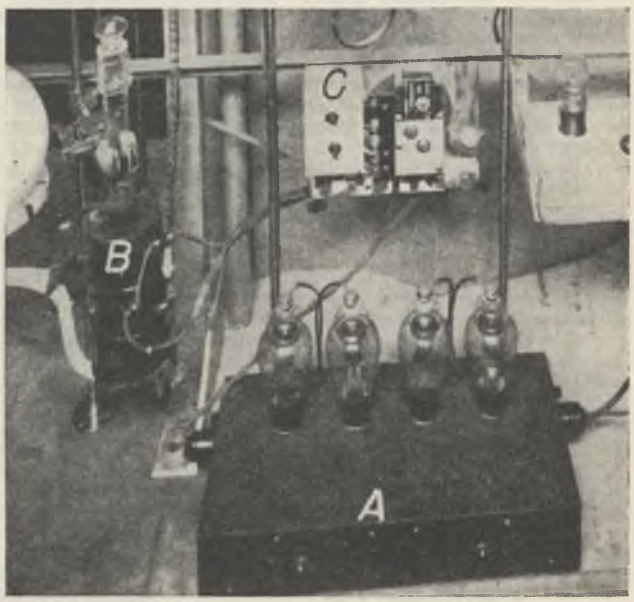

Figure 1. Solenoid Pump and Accessory Apparatus

The side arm, A, connects the intake to the pump cylinder at a point just below the maximum height reached by the top of the plunger in its upstroke. This arrangement permits any liquid that leaks by the plunger during pumping to return directly to the intake for repumping and at the same time relieves the back pressure on the plunger. The two valves, V_1 and V_2, control the flow of the liquid through the pump. Each valve is composed of a ground-glass plate, D, approximately 1 mm. thick, which makes contact with the ground end of a tube projecting into the valve chamber, C. The side wall of the valve chamber is indented in three places in order to keep the ground-glass plate in a vertical position close to the end of the projecting tube, so that proper valve action may be attained. The valves are tilted slightly from the horizontal to facilitate the draining of the pump and connecting lines through the stopcock, E.

Three direct current solenoids, S_{1-3} (Cutler-Hammer coils Nos. 9, 91, 55, 115-volt direct current, Cutler-Hammer, Inc., Detroit, Mich.), spaced to give the desired stroke, are used to operate the plunger. S_1 raises the plunger and holds it until the pump cylinder is filled, then is automatically removed from the circuit by the flasher. Simultaneously, S_2 and S_3, connected in parallel, are energized and pull the plunger on the downward pumping stroke. The speed of the flasher motor, through which the direct current to the solenoids is controlled, may be varied when necessary to give a range of 7 to 30 complete pumping cycles per minute. The best pumping cycle allows the barrel of the pump just enough time to fill, following the practically instantaneous upstroke and prior to the beginning of the downward stroke. The time for a complete cycle of the piston, when a vegetable oil at 50° C. was pumped through an 8-mm. tube to a reservoir approximately 240 cm. (8 feet) above the valves, was 4 seconds. The 4-second cycle may be divided into 2.5 seconds for the upstroke and filling of the pump, and 1.5 seconds for the downward stroke.

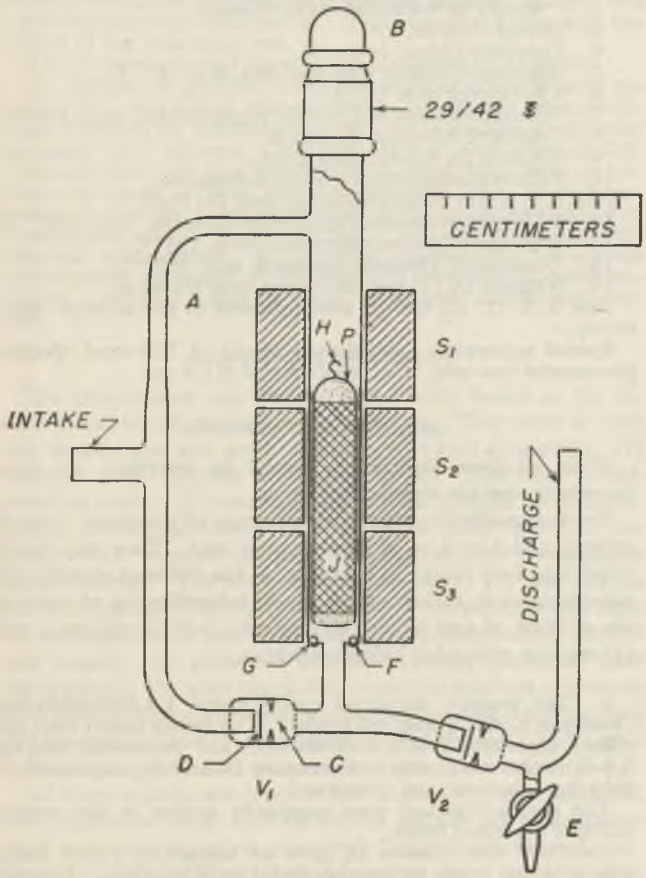

Figure 2. Detail of Pump Construction

The pump valves and connecting lines may be wrapped with resistance wire in order to heat the more viscous liquids, like hydrogenated fats, to facilitate their flow.

Power Supply. A full-wave bridge rectifier was used to energize solenoids S_1, S_2, and S_3. Four Type 866A/866 mercury-vapor tubes were employed in the network, and their electronic output was unfiltered. The wiring diagram and description of the components appear in Figure 3.

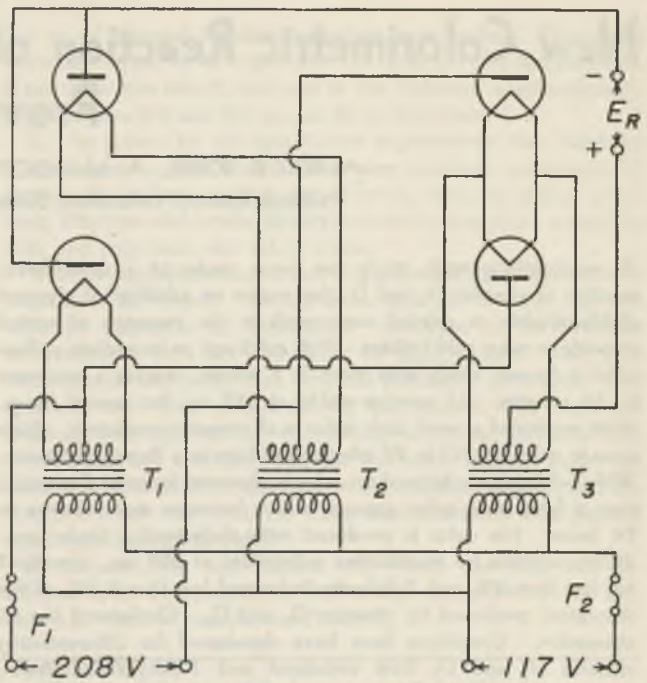

Figure 3. Circuit Diagram for 866 Bridge Rectifier

F_1. Fuse, 1 ampere
F_2. Fuse, 3 amperes
T_1, T_2. Filament transformer, 2.5 volts, 5 amperes, Thordarson T19F88
T_3. Filament transformer, 2.5 volts, 10 amperes, Thordarson T19F

The bridge network was chosen in preference to other rectifier circuits because of its simplicity, and because there were available no commercially built transformers that could supply 500 milliamperes at 150 to 200 volts after rectification. This type of rectifier has the advantage of developing an average direct current voltage that is nearly 90% of the effective alternating current input voltage, when the voltage drop through the tube is disregarded.

In the application described, the plates of the tubes were supplied with 208 volts alternating current from the laboratory line. The average direct current voltage developed by the rectifier was 160 volts. The network can easily supply 500 milliamperes, and has given entirely satisfactory service during more than a year of operation.

A voltage-doubler circuit (*3*) operating from a 117-volt alternating current supply might prove practical as a power source for the pump in laboratories having no 208-volt line.

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Ohio Valley Spectrographic Society

Representatives of approximately fifteen industrial and educational laboratories in southwestern Ohio, meeting in Dayton, Ohio, have formed the Ohio Valley Spectrographic Society. The group is interested in promoting interest in and knowledge of spectrographic analysis. Meetings will be held every six weeks in Cincinnati, Columbus, Dayton, or Middletown, Ohio. Information concerning the new organization may be obtained from Miss B. J. Beisel, Materials Laboratory, National Cash Register Co., Dayton 9, Ohio.

New Colorimetric Reaction of Vitamins D_2 and D_3 and Their Provitamins

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A spectrophotometric study has been made of a colorimetric reaction of vitamins D_2 and D_3 that occurs on addition of glycerol dichlorohydrin or related compounds in the presence of acetyl chloride or other acid halides. With calciferol an immediate yellow color is formed which turns green in 1 minute, reaches a maximum in 15 minutes, and remains stable at 625 mμ for several hours. With ergosterol a weak pink color is observed immediately, which turns to orange in 15 to 20 minutes and later to a fluorescent green. With 7-dehydrocholesterol no color is observed for several minutes, then a faint pink color appears, which becomes more intense in 24 hours. No color is produced with cholesterol. Under conditions suitable for quantitative estimations at 625 mμ, ergosterol has less than 4% and 7-dehydrocholesterol less than 0.3% of the absorption produced by vitamins D_2 and D_3. Cholesterol has no absorption. Conditions have been developed for differentiating vitamins D_2 and D_3 from ergosterol and 7-dehydrocholesterol, ergosterol from 7-dehydrocholesterol, and the dinitrobenzoate esters of these compounds from the free sterols.

THE investigation reported in this paper deals with a spectrophotometric study of a new colorimetric reaction of vitamins D_2 and D_3 and related sterols (which was observed by the senior author, A. E. Sobel). This reaction is given on the addition of glycerol dichlorohydrin or related compounds in the presence of acetyl chloride or other halides of acid nature.

Under conditions which might be suitable for quantitative estimations of vitamins D_2 and D_3 (at 625 mμ), ergosterol has less than 4% and 7-dehydrocholesterol less than 0.3% of the absorption produced by vitamins D_2 and D_3. Cholesterol has no absorption at all. Qualitatively one can readily differentiate (1) vitamins D_2 and D_3 from ergosterol and 7-dehydrocholesterol and (2) ergosterol from 7-dehydrocholesterol. To the authors' knowledge this is the first colorimetric reaction that distinguishes between ergosterol and 7-dehydrocholesterol. Conditions were found which permit differentiation between the dinitrobenzoate esters of the above compounds and the free sterols.

The hitherto available colorimetric reactions given by vitamin D have been reviewed by Ewing *et al.* (*1*), who applied the antimony trichloride reaction of Nield *et al.* (*4*) to the estimation of vitamin D in fish oils. This reaction when compared to the authors' reaction has the advantage of a higher extinction coefficient at the wave length of maximum light absorption. The advantages of the authors' reaction over the antimony trichloride are:

(1) the colors produced are relatively stable, (2) the color-producing reagent, glycerol dichlorohydrin, is stable, and no special technique is involved in its preparation and application. (3) The shape of the absorption spectra is entirely different for vitamin D, ergosterol, and 7-dehydrocholesterol, the maximum being at 625 mμ for vitamin D and between 500 and 525 mμ for the two provitamins. With antimony trichloride these maxima are very close together near 500 mμ. (4) This reaction is more specific for vitamin D than antimony trichloride because there is no reaction with cholesterol, less interference produced by 7-dehydrocholesterol at 625 mμ, and less interference produced by ergosterol at 625 mμ.

This last statement requires further elucidation. Table II of the paper of Ewing *et al.* (*1*) indicates that there is a 13 to 26% increase in the extinction coefficient due to the addition of ergosterol in amounts slightly higher than the vitamin D originally present. There is a contradiction between these results and those obtained by Nield *et al.* (*3*, *4*), who mentioned an extinction coefficient of 42 for cholesterol and 25 for ergosterol compared to 1800 for calciferol. If we accept Nield's results the interference of ergosterol is less than in the authors' reaction.

The color reaction for vitamin D with pyrogallic acid described by Halden and Tzoni (*2*) is not given by ergosterol and 7-dehydrocholesterol, according to these authors. This reaction though reported in 1936, has not found favor because of the inherent difficulties involved in standardizing the heating and preparation of the aluminum chloride solution in alcohol. One of the present authors (A. E. Sobel) found the reaction very difficult to control.

REAGENTS AND APPARATUS

1. Chloroform, C.P., Mallinckrodt.
2. Glycerol 1,3-dichlorohydrin, Eastman Kodak practical grade (colorless liquid). If liquid is not colorless it should be redistilled under vacuum.
3. Acetyl chloride, C.P.
4. Calciferol (Mead Johnson).
5. Ergosterol, freshly recrystallized, m.p. 162° C.
6. Cholesterol, m.p. 147° C.
7. 7-Dehydrocholesterol, freshly recrystallized, m.p. 150° C.
8. Calciferol 3,5-dinitrobenzoate.
9. Vitamin D_3 3,5-dinitrobenzoate.
10. 7-Dehydrocholesterol 3,5-dinitrobenzoate.

Nos. 7, 8, 9, and 10 were obtained from Du Pont.

11. 7-Dehydrocholesterol 3,5-dinitrobenzoate, m.p. 208° C.
12. Ergosterol 3,5-dinitrobenzoate, m.p. 198° C.
13. Cholesterol 3,5-dinitrobenzoate, m.p. 187° C.
14. Vitamin D_3 (Ayerst, McKenna, and Harrison).

Nos. 5, 6, 11, 12, and 13 were prepared in the authors' laboratory.

Special apparatus. A Coleman Model 11 Universal spectrophotometer was used, with cell diameter of 1.3 cm.

QUALITATIVE EXPERIMENTS

While all these experiments cannot be described, the more important ones are summarized below.

For the qualitative experiments a trace of the sterol, 1 cc. of solvent, and 1 or 2 cc. of reagent were used. They may be divided into four parts: (1) reaction of the different sterols; (2) color reaction in various solvents; (3) intensification of color by the addition of acid halides to glycerol 1,3-dichlorohydrin; and (4) reaction with other halogenohydrins.

1. The reagent employed was glycerol 1,3-dichlorohydrin (Eastman Kodak's practical grade). The sterols tested were calciferol, ergosterol, 7-dehydrocholesterol, and cholesterol, and the 3,5-dinitrobenzoate esters of vitamins D_2 and D_3, ergosterol, 7-dehydrocholesterol, and cholesterol.

The results obtained were essentially similar in the various solvents described below.

Calciferol and vitamin D_3 gave an immediate yellow color, which turned green within the first 1 or 2 minutes; the color intensified considerably in the first 20 minutes, while a slight increase in color was still noticeable during the next 24 hours. The 3,5-dinitrobenzoates of vitamins D_2 and D_3 gave the same reaction as calciferol, only somewhat slower.

Ergosterol at first gave no visible color. In about 2 to 3 minutes a pink color appeared, which turned to orange after about 30 minutes, and to a green fluorescent color during the next 2 to 3 hours. After this period no further change was observed.

Ergosterol 3,5-dinitrobenzoate behaved like the free ergosterol. In the case of 7-dehydrocholesterol at first no visible color was observed. A faint pink color was noticeable after about 1 hour,

which increased only slightly within 24 hours. Cholesterol and the 3,5-dinitrobenzoates of 7-dehydrocholesterol and cholesterol showed no color in the first 24 hours. In about 2 to 3 days a very faint greenish color was noticeable.

2. The reactions of glycerol 1,3-dichlorohydrin with the sterols, dissolved in various solvents, are described below. A green color with calciferol developed in chloroform, carbon tetrachloride, ethylene dichloride, acetylene tetrachloride, benzene, glacial acetic acid, dibutyl phthalate, and petroleum and ethyl ethers, where two layers were formed, the lower one green.

No color developed in 95% alcohol, acetone, acetic anhydride, a mixture of acetic anhydride and chloroform, a mixture of acetic anhydride and glacial acetic acid, propylene glycol, Cellosolve, triethanolamine, morpholine, dioxane, pyridine, ethylene chlorohydrin, trimethylene chlorohydrin, propylene chlorohydrin, glycerol α-monochlorohydrin, propionic anhydride, and butyric anhydride. The reactions of the various sterols are described above.

3. Upon purification by filtration over charcoal or by redistillation, glycerol 1,3-dichlorohydrin no longer gave a color with any of the sterols mentioned. On addition of various acidic halides the reagent regained its activity. An immediate green color was obtained with calciferol upon the addition of hydrochloric acid (liquid or anhydrous), a mixture of hydrochloric and sulfuric acids, hydrobromic acid, acetyl chloride, phosphorus pentachloride, phosphorus oxychloride, or benzoyl chloride. A slowly developing green color appeared upon the addition of concentrated sulfuric acid, barium chloride, aluminum chloride (solution cloudy, probably owing to a precipitation of aluminum hydroxide). A rusty brown color developed with stannic chloride, a pale orange color with mercuric chloride. No color formed on the addition of phosphorus trichloride, stannous chloride, zinc chloride, or thionyl chloride. When thionyl chloride was added the green color (formed between calciferol and 1,3-dichlorohydrin) disappeared. This may offer a clue as to the nature of the color produced.

All the sterols showed the reactions described in (1) above.

4. Instead of glycerol 1,3-dichlorohydrin other related reagents were also tested with calciferol. Under the same conditions described for 1,3-dichlorohydrin, an immediate green color appeared with glycerol 1,3-dibromohydrin and glycerol 2,3-dichlorohydrin. A slow reaction took place with 1-chloro-2,3-dibromopropane. These reagents behaved like 1,3-dichlorohydrin, in that the color became more intense upon the addition of hydrochloric acid or acetyl chloride. No color developed with ethylene chlorohydrin, trimethylene chlorohydrin, propylene chlorohydrin, propylene glycol, acetylene tetrachloride, trichlorohydrin, 1 chloro-2,3-epoxypropane, or glycerol α-monochlorohydrin.

QUANTITATIVE EXPERIMENTS

The quantitative experiments were mainly based on the results found in the qualitative experiments. They were divided into three major and seven minor parts, which consisted of (I) reaction with glycerol 1,3-dichlorohydrin (Eastman Kodak practical grade); (II) reaction with purified glycerol 1,3-dichlorohydrin, containing anhydrous hydrochloric acid; and (III) reaction with purified glycerol 1,3-dichlorohydrin, containing acetyl chloride. The seven subdivisions were: (1) best solvent for development of color; (2) influence of temperature and light on development of color; (3) preferable proportions of solvent and reagent; (4) period of time for reaching maximum color development; (5) wave length best suited for readings of vitamin D in spectrophotometer; (6) relationship between concentration and absorption; and (7) best proportions of glycerol 1,3-dichlorohydrin and hydrochloric acid or acetyl chloride.

All these experiments were at first carried out with calciferol. The conditions which were considered suitable for quantitative work were then tested on ergosterol, 7-dehydrocholesterol, cholesterol, and the 3,5-dinitrobenzoates of the above sterols in addition to the dinitrobenzoates of vitamins D_2 and D_3. The original reason for testing the dinitrobenzoates was inability to obtain pure vitamin D_3. Later on the authors were able to obtain crystalline vitamin D_3 but because of the limited amount available,experiments were not so exhaustive as with the other sterols tested.

I. Reactions with Practical Glycerol 1,3-Dichlorohydrin. For the experiments described in (1) and (2) below 0.3 mg. of calciferol was dissolved in 3 cc. of solvent and to this 1.5 cc. of glycerol 1,3-dichlorohydrin were added. The colors were developed for 45 minutes in the dark at room temperature, if not otherwise stated, and read in the Coleman spectrophotometer between 400 and 650 mμ, at 25-mμ intervals.

1. As shown by the qualitative experiments, the following solvents gave a color reaction and were therefore quantitatively tested: chloroform, carbon tetrachloride, benzene, glacial acetic acid, ethylene dichloride, dibutylphthalate, acetylene tetrachloride, and petroleum and ethyl ethers.

With dibutylphthalate, carbon tetrachloride, and benzene very little color developed. Glacial acetic acid did not mix too well with the reagent in all proportions. As ethers are very volatile solvents, they were not well suited for this purpose. Acetylene tetrachloride and even more ethylene dichloride gave good color reactions with some proportions of the reagent, but could not compare with chloroform. Chloroform was found to be the best solvent for this reaction, because the most intense color was reached with it in all proportions of solvent and reagent, and the straightest line was obtained when concentrations were plotted against absorption of light.

2. Experiments were carried out, where the color was developed in the refrigerator, at room temperature (26° to 28° C.), and in 37° and 60° incubators (Table I).

Table I. Influence of Temperature on Color Development

(Calciferol dissolved in chloroform and treated with glycerol 1,3-dichlorohydrin)

Log I_0/I at 625 mμ

Refrigerator	Room temperature, 27° C.	37° C.	60° C.
0.014	0.098	0.104	0.096

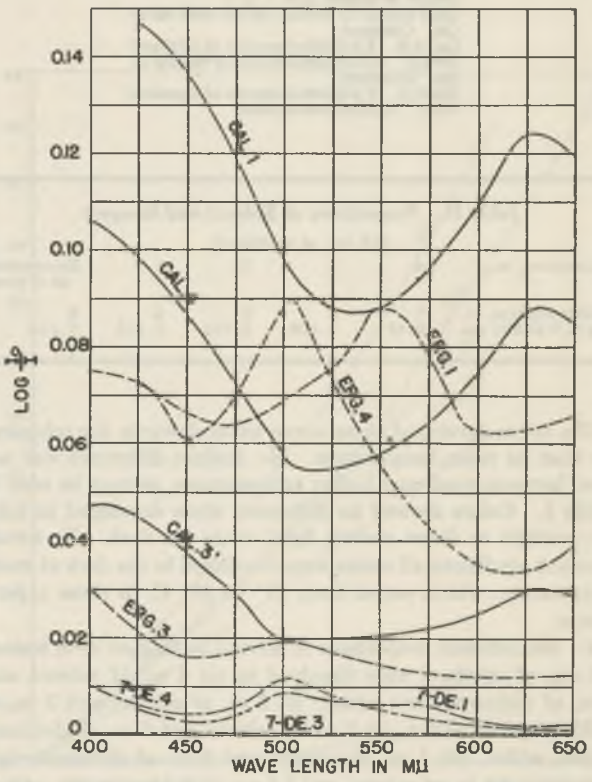

Figure 1. Absorption Spectra of Sterols

Conditions	Glycerol 1,3-Dichlorohydrin	Solvent to Reagent Ratio	Calciferol	Ergosterol, 7-Dehydrocholesterol
			Mg.	*Mg.*
1	Practical	1:4	0.3	3.0
3	0.5% acetyl chloride	4:1	0.2	2.0
4	1.0% acetyl chloride	3:2	0.2	2.0

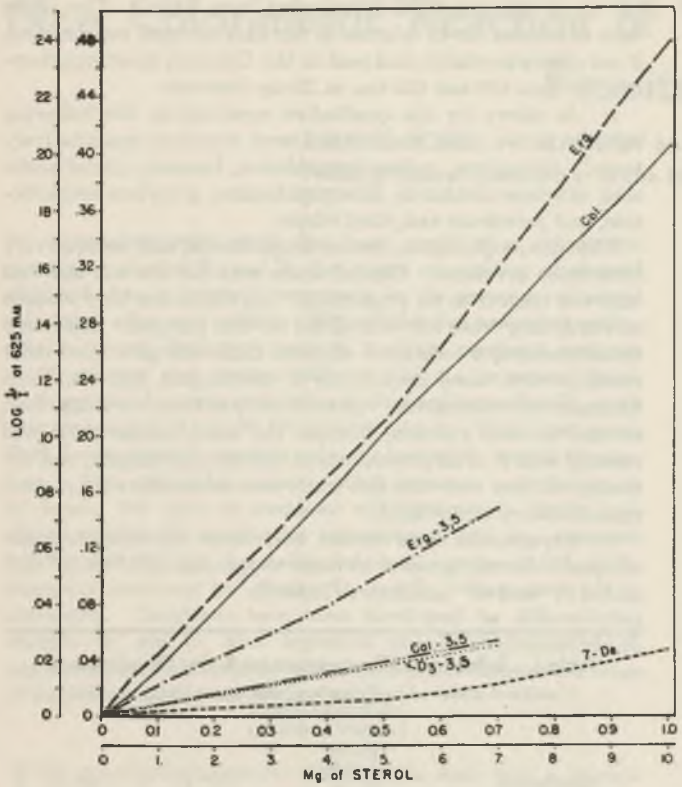

Figure 2. Relation between Concentration of Sterols and Light Absorption

Solvent to reagent ratio, 1 to 4
Only vitamin D plotted against inner scales
Cal. Calciferol
Cal.-3,5. 3,5-Dinitrobenzoate of calciferol
D_3-3,5. 3,5-Dinitrobenzoate of vitamin D_3
Erg. Ergosterol
Erg.-3,5. 3,5-Dinitrobenzoate of ergosterol
7-De. 7-Dehydrocholesterol

Table II. Proportions of Solvent and Reagent

(0.3 mg. of calciferol)

Chloroform, cc.	4	3	2	1	Evaporated to dryness
Dichlorohydrin, cc.	1	2	3	4	5
Log I_0/I at 625 mμ	0.071	0.109	0.111	0.118	0.112

The colors developed about seven times slower in the refrigerator than at room temperature. No distinct difference was noticed between room and higher temperatures, as may be seen in Table I. Colors showed no difference when developed in light (no sunlight or direct electric light) or in the dark. To assure constant conditions all colors were developed in the dark at room temperature, which varied from 26° to 28° C. in these experiments.

3. Six different proportions of solvent to reagent were tested: 0.3 mg. of calciferol were dissolved in (*a*) 4 cc. of solvent and 1 cc. of dichlorohydrin added, (*b*) 3 cc. of solvent and 2 cc. of dichlorohydrin added, (*c*) 2 cc. of solvent and 3 cc. of dichlorohydrin added, (*d*) 1 cc. of solvent and 4 cc. of dichlorohydrin added, (*e*) 0.5 cc. of solvent and 4.5 cc. of dichlorohydrin added, and (*f*) solvent evaporated to dryness and 5 cc. of dichlorohydrin added.

In this series of experiments the colors were developed for 30 minutes in the dark at room temperature and read in the spectrophotometer at wave lengths between 400 and 650 mμ, at 25-mμ intervals.

Even though chloroform seemed to be the best solvent, these experiments were carried out with all the above-mentioned solvents.

Maximum absorption was obtained when calciferol was dissolved in 1 cc. of chloroform and 4 cc. of dichlorohydrin were added (Table II). In all solvents tested the maximum color was obtained with the same proportion of solvent and reagent.

4. To find the time of maximum absorption experiments were carried out using 0.3 mg. of calciferol in 3, 2, and 1 cc. of solvent and the volume was made to 5 cc. with dichlorohydrin. The solvents used were chloroform, benzene, glacial acetic acid, carbon tetrachloride, and ethylene dichloride. The colors were read in the spectrophotometer between 400 and 650 mμ after developing them in the dark at room temperature for 5, 10, 15, 20, 30, 45, 60, and 90 minutes, and 2, 3, 4, and 6 hours.

After the first 15 minutes had passed the intensity of the color remained almost constant with only a slight rise in the absorption afterward (Table III).

Based on these results, at first, 30 minutes were chosen for color development but as ergosterol interfered more in this period of time (as described below) the colors of calciferol were read at the end of 15 minutes.

5. The previous experiments indicated the most suitable wave length to be employed. Additional experiments were carried out to ascertain the wave length of light most suited for calciferol studies. The same three proportions of chloroform and dichlorohydrin were taken as described in (3). Colors were developed for 30 minutes in the dark at room temperature. The wave length of maximum absorption of calciferol was reached in the authors' spectrophotometer, which does not go beyond 400 mμ, at 400 to 425 mμ. A second peak was reached at 625 mμ, which was chosen as the most convenient wave length, since it is the wave length of minimum absorption of ergosterol and 7-dehydrocholesterol.

Typical absorption spectra of calciferol, ergosterol, and 7-dehydrocholesterol are shown in Figure 1, which was taken under the best conditions for differentiating calciferol from the other sterols. There is a maximum for calciferol at 625 mμ and in the ultraviolet beyond 400, and a minimum between 500 and 550 mμ. There is a maximum at 550 for ergosterol and at 500 for 7-dehydrocholesterol and a minimum for ergosterol and 7-dehydrocholesterol at 625 and 450. The shapes of the absorption spectra of the 3,5-dinitrobenzoates were similar to those of the free sterols.

6. The relationship between concentration of sterols and light absorption is shown in Figure 2. The sterols were dissolved in 1 cc. of chloroform and the color was developed by adding 4 cc. of glycerol 1,3-dichlorohydrin and allowing the solution to stand in the dark at room temperature for 20 minutes. The light absorption was read at the end of this time at 625 mμ. This time was chosen because calciferol reached its maximum absorption, but the other sterols did not and thus the difference was most marked.

Table III. Influence of Time on Color Development

(0.3 mg. of calciferol in 1 cc. of solvent + 4 cc. of dichlorohydrin)

	Log I_0/I at 625 mμ							
Solvent	5 min.	10 min.	15 min.	20 min.	30 min.	60 min.	2 hours	6 hours
$CHCl_3$	0.064	0.093	0.118	0.121	0.121	0.122	0.132	0.142
Benzene	...	...	0.078	0.084	0.089	0.090	0.102	...
CH_3COOH	...	...	0.080	0.088	0.091	0.102	0.112	...
CCl_4	...	...	0.069	0.076	0.080	0.090	0.111	...
Ethylene dichloride	...	...	0.072	0.088	0.092	0.098	0.112	...

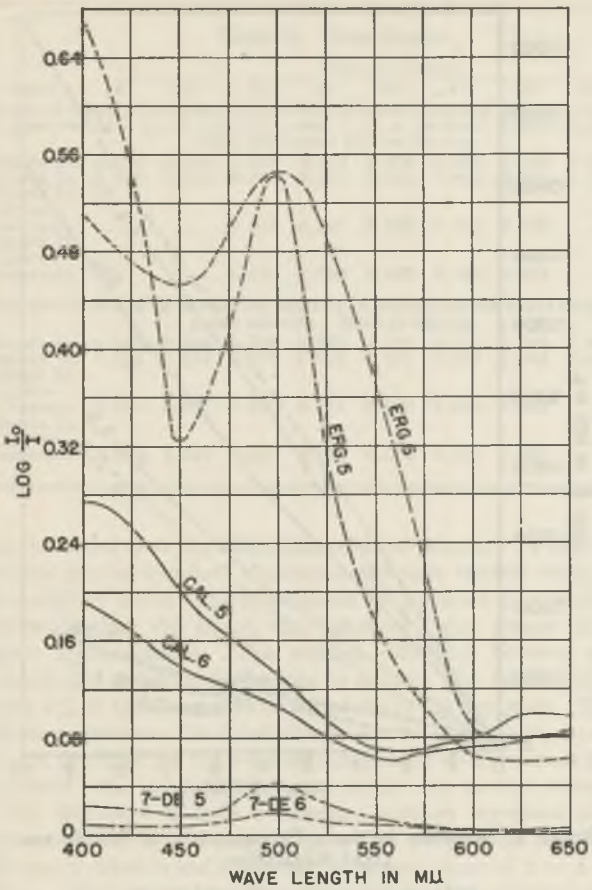

Figure 3. Absorption Spectra of Sterols

Conditions	Glycerol 1,3-Dichlorohydrin	Solvent to Reagent Ratio	Calciferol *Mg.*	Ergosterol, 7-Dehydrocholesterol *Mg.*
5	4% acetyl chloride	2:3	0.2	2.0
6	2% acetyl chloride	1:4	0.2	2.0

It is seen in Figure 2 that under these conditions there is a marked difference between the free sterols and the dinitrobenzoates. The dinitrobenzoate of calciferol has about one sixth of the absorption of the free vitamin. The dinitrobenzoate of 7-dehydrocholesterol gives no color at all. The dinitrobenzoate of ergosterol has about one half the absorption of the free sterol.

The second point worth mentioning is the marked difference in the colors produced by ergosterol and 7-dehydrocholesterol. This applies to the free sterols as well as to the dinitrobenzoates. This is in marked contrast to the two vitamins derived from these sterols, whose absorptions differ only to a minor degree.

II. Reactions with Pure Glycerol 1,3-Dichlorohydrin Containing Anhydrous Hydrochloric Acid. The reagent was prepared by passing freshly dry generated hydrochloric acid directly into glycerol 1,3-dichlorohydrin. The saturated solution was approximately 0.75 *N*. In addition to the six experiments described under I, a seventh was added to determine the influence of various concentrations of hydrochloric acid in the dichlorohydrin reagent. The different proportions tested were (*a*) 15 parts of pure dichlorohydrin and 1 part saturated with hydrochloric acid, (*b*) 9 parts of pure reagent and 1 part saturated with hydrochloric acid, (*c*) 4 parts of pure reagent and 2 parts saturated with hydrochloric acid, and (*d*) dichlorohydrin completely saturated with hydrochloric acid. The last gave the most color. The best proportion of solvent to reagent was also 1 to 4. When anhydrous hydrochloric acid was added to the solvent, no improvement of the results was noticed. The solvent became yellowish and the color intensity produced on addition of the reagent was not reproducible.

The colors obtained with the hydrochloric acid reagent were more intense than the ones obtained with practical dichlorohydrin alone. Ergosterol gave about the same reaction as with practical dichlorohydrin, the intensity of the color being somewhat greater.

The investigations on hydrochloric acid as agent to intensify the color were not completed, as it was noticed that the results obtained were not very reproducible, though the amount of hydrochloric acid present in the reagent was determined on each batch of fresh reagent and was found to be fairly constant.

III. Reactions with Pure Glycerol 1,3-Dichlorohydrin Containing Acetyl Chloride. The experiments with acetyl chloride are described in detail, since the color produced with dichlorohydrin in the presence of acetyl chloride seems well suited for quantitative purposes. These colors are more intense than those obtained under the previously described conditions. The results obtained on the spectrophotometer were reproducible as much as 6 months apart, employing different batches of calciferol and acetyl chloride. The acetyl chloride employed was at first redistilled but this was found to be a needless precaution.

Here again chloroform seemed to be the medium in which the most intense colors were produced between calciferol and the reagent. (When the chloroform was specially purified and redistilled, the colors were about 20% more intense. Chloroform without any treatment was actually employed, since the specially purified chloroform deteriorates on standing.)

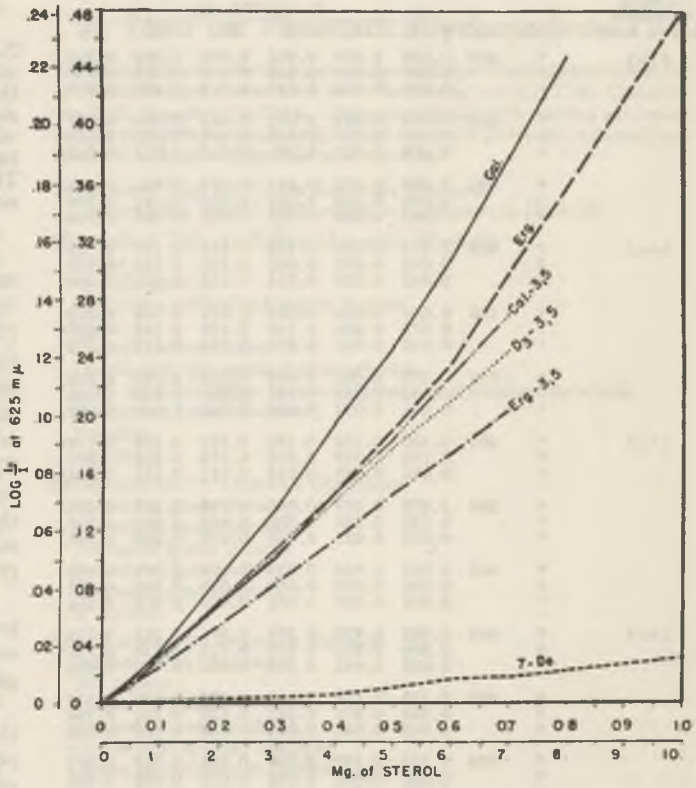

Figure 4. Relation between Concentration of Sterols and Light Absorption

Dichlorohydrin containing 1% acetyl chloride
Solvent to reagent ratio, 3 to 2
Only vitamin D plotted against lower scales

The results were obtained with 0.2 mg. of calciferol, 2.0 mg. of ergosterol, and 2.0 mg. of 7-dehydrocholesterol and read at the end of 15 minutes. The ratios of solvent to reagent explored were 4 to 1, 3 to 2, 2 to 3, and 1 to 4. The acetyl chloride in the reagent varied from 0.5 to 5% by volume.

The shape of the absorption spectrum underwent a change with the various proportions of reagent. The percentage of acetyl chloride affected only the intensity but not the shape, the maximum for calciferol at 625 mμ being more distinct with low proportions of reagent whereas the maximum at 400 becomes rapidly more distinct with higher proportions of the reagent. This becomes even more evident on examining the minimum between 525 and 550 mμ. There is a greater difference between the absorption minimum and 625 mμ at the lower proportion of reagent, and between 400 mμ and the minimum at the higher proportions of reagent. The actual minima at the higher proportions of reagent are at 550 mμ and there is a distinct rise in absorption by 500 mμ. These changes may be seen in the representative Figures 3 and 4.

The results at 400, 500, and 625 mμ are given in Table IV. Each figure is the average of two to four estimations, which were all close together and were shown to be reproducible months later. Ten times as much ergosterol and 7-dehydrocholesterol was used as calciferol. By looking at these tables one can choose the conditions suitable for various purposes.

For example, with 0.5% of acetyl chloride and a solvent-reagent ratio of 4 to 1 7-dehydrocholesterol gives no color at 625 mμ and is thus well suited for determining ergosterol or calciferol in the presence of 7-dehydrocholesterol. At a ratio of 3 to 2 and 1% acetyl chloride at 625 mμ ergosterol has less than 4% and 7-dehydrocholesterol less than 0.3% of the color intensity of calciferol. These conditions may be used for evaluating calciferol in the presence of the other two sterols. The other sterols, especially ergosterol, may be evaluated under the conditions given with a ratio of 2 to 3 and 4% acetyl chloride at 500 mμ. Thus by two determinations it may be possible to evaluate both sterols. The same thing might be accomplished at a ratio of 1 to 4 and 2% acetyl chloride.

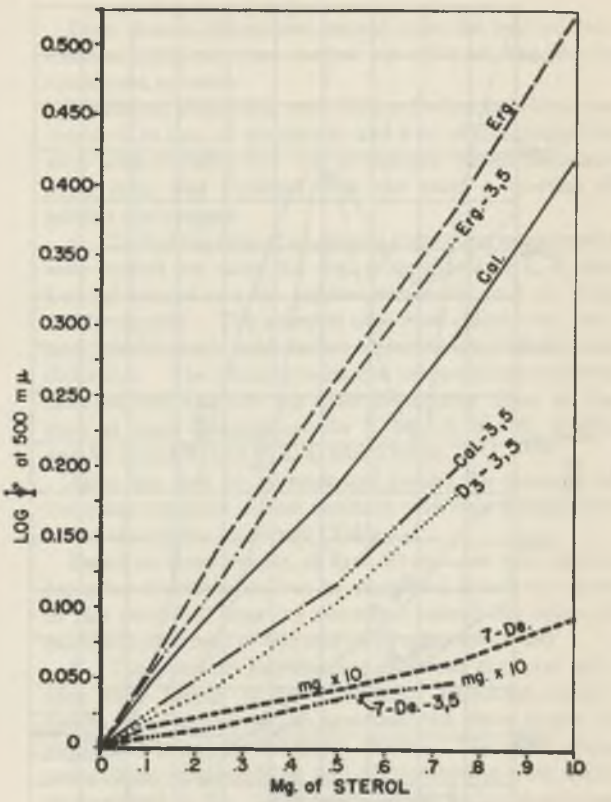

Figure 5. Relation between Concentration of Sterols and Light Absorption

Dichlorohydrin containing 4% acetyl chloride
Solvent to reagent ratio, 2 to 3

Table IV. Influence of Acetyl Chloride on Color Development after 15 Minutes

$CHCl_2$-Dichlorohydrin Ratio	Sterol	mμ	Acetyl Chloride 0.5%	1%	2%	3%	4%	5%
4 to 1	a	400	0.048	0.058	0.059	0.068	0.088	0.052
	b		0.030	0.050	0.048	0.072	0.055	0.045
	c		0.000	0.033	0.010	0.018	0.010	0.008
	a	500	0.020	0.028	0.031	0.041	0.040	0.031
	b		0.020	0.037	0.043	0.068	0.065	0.055
	c		0.000	0.017	0.005	0.012	0.012	0.010
	a	625	0.030	0.035	0.041	0.044	0.048	0.041
	b		0.014	0.023	0.025	0.038	0.031	0.029
	c		0.000	0.008	0.002	0.008	0.007	0.004
3 to 2	a	400	0.075	0.106	0.105	0.117	0.127	0.125
	b		0.055	0.075	0.085	0.102	0.125	0.105
	c		0.000	0.019	0.014	0.012	0.006	0.006
	a	500	0.051	0.058	0.058	0.072	0.078	0.073
	b		0.071	0.092	0.120	0.178	0.185	0.160
	c		0.010	0.015	0.018	0.023	0.017	0.013
	a	625	0.073	0.088	0.089	0.088	0.082	0.034
	b		0.034	0.034	0.047	0.055	0.055	0.060
	c		0.004	0.003	0.006	0.009	0.003	0.003
2 to 3	a	400	0.130	0.170	0.190	0.192	0.192	0.110
	b		0.128	0.230	0.282	0.378	0.518	0.370
	c		0.005	0.020	0.014	0.011	0.011	0.008
	a	500	0.078	0.079	0.095	0.098	0.107	0.065
	b		0.152	0.265	0.338	0.462	0.552	0.372
	c		0.015	0.022	0.025	0.028	0.020	0.015
	a	625	0.088	0.098	0.098	0.091	0.078	0.063
	b		0.051	0.069	0.068	0.080	0.081	0.071
	c		0.004	0.004	0.005	0.009	0.003	0.004
1 to 4	a	400	0.262	0.275	0.278	0.265	0.247	0.218
	b		0.306	0.545	0.672	0.725	0.735	0.712
	c		0.025	0.041	0.025	0.020	0.019	0.009
	a	500	0.128	0.110	0.132	0.133	0.122	0.113
	b		0.285	0.375	0.542	0.520	0.456	0.149
	c		0.028	0.040	0.041	0.028	0.028	0.028
	a	625	0.116	0.102	0.098	0.085	0.075	0.072
	b		0.065	0.066	0.062	0.070	0.068	0.067
	c		0.009	0.008	0.007	0.008	0.008	0.008

[a] Calciferol, 0.2 mg.
[b] Ergosterol, 2.0 mg.
[c] 7-Dehydrocholesterol, 2.0 mg.
Values expressed as log I_0/I.

The conditions found best for the differentiation of calciferol and ergosterol were applied to the dinitrobenzoates of calciferol, vitamin D_3, ergosterol, 7-dehydrocholesterol, and cholesterol. (These were 3 to 2 solvent to reagent ratio and 1% acetyl chloride in reagent.) 7-Dehydrocholesterol and cholesterol 3,5-dinitrobenzoate gave no color at all in amounts as high as 10 mg. of sterols. In contrast to this, both ergosterol and the two vitamins gave a distinct color similar to that of the free sterols. Thus the 3,5-dinitrobenzoate ester serves as an excellent method for the differentiation of 7-dehydrocholesterol from ergosterol and the two forms of vitamin D, especially so, as the esters of those sterols give almost as great an amount of color as the free sterols (Figure 4).

This is in marked contrast to the color developed with only practical 1,3-dichlorohydrin (Figure 2), where the dinitrobenzoate esters containing corresponding amounts of free sterols gave only about 20% as much color as the free vitamins.

The shapes of the absorption curves were essentially those of the free sterols. Quantitatively there was less absorption per unit weight of sterol, the difference being greater with increasing concentrations of test substance (Figure 4).

The relation between absorption (as expressed by log I_0/I at 625 mμ) and concentration of sterol is presented in Figure 4. The conditions employed were the same, 1% acetyl chloride in reagent and a 3 to 2 ratio of solvent to reagent. In Figure 4 it

Table V. Time Studies

Sterol	Time in Minutes 5	10	15	20	30	45	60	120	300
0.2 mg. of calciferol, vitamin D_3, 3,5-dinitrobenzoate esters of calciferol, and vitamin D_3 dissolved in 1 cc. of chloroform and 4 cc. of practical dichlorohydrin added. Light absorption read at 625 mμ									
Vitamin D_2	0.026	0.058	0.076	0.078	0.078	0.079	0.080	0.085	0.088
Vitamin D_3	0.005	0.016	0.031	0.043	0.062	0.084	0.099	0.112	0.113
Vitamin D_2 dinitrobenzoate	...	...	0.017	0.027	0.033	0.035	0.036	...	...
Vitamin D_3 dinitrobenzoate	...	...	0.015	0.026	0.032	0.035	0.038	...	...
Same sterols in 3 cc. of chloroform and 2 cc. of dichlorohydrin added containing 1% acetyl chloride. Read at 625 mμ									
Vitamin D_2	0.046	0.068	0.086	0.087	0.087	0.088	0.088	0.090	0.090
Vitamin D_3	0.038	0.042	0.074	0.073	0.071	0.067	0.062	0.062	0.065
Vitamin D_2 dinitrobenzoate	0.034	0.055	0.070	0.078	0.080	0.080	0.082	...	...
Vitamin D_3 dinitrobenzoate	0.034	0.048	0.065	0.074	0.079	0.078	0.077	...	..

may be noted that the dinitrobenzoates of vitamins D_2 and D_3 produce almost identical absorption although that of vitamin D_3 is slightly lower. The free vitamin D_2, however, has a higher absorption than the esters, the difference being greater with higher concentrations. The marked difference between ergosterol and 7-dehydrocholesterol is evident, the latter giving about 6% of the absorption of ergosterol in the free state. The difference between the 3,5-dinitrobenzoates is absolute, as no color is produced by the 7-dehydrocholesterol ester, whereas the ergosterol ester produces almost as much color as free sterol.

The difference in light absorption between ergosterol and 7-dehydrocholesterol is emphasized where the reagent contains 4% acetyl chloride and a solvent to reagent ratio of 2 to 3 at 500 mμ. The relations between absorption and concentration are given in Figure 5. Under these conditions 7-dehydrocholesterol 3,5-dinitrobenzoate gives some absorption, but less than the free sterol. Cholesterol still does not produce any color.

INVESTIGATIONS ON VITAMIN D_3

Investigations with vitamin D_3 were limited, because of inability to obtain more than relatively small amounts of this compound. The specimen obtained proved to be very unstable, in marked contrast to the other sterols, including the 3,5-dinitrobenzoate ester of vitamin D_3 and calciferol. The results with these specimens of vitamin D_3 were not so reproducible as those with the other compounds described. Whether this is an inherent property of vitamin D_3 or due to small impurities in the sample will require further investigation.

In Table V the results of time studies are given under two sets of conditions. The rates of color development of vitamins D_2 and D_3 are different under the same set of conditions. In contrast to this, the 3,5-dinitrobenzoates of these compounds behave alike.

The shape of the absorption spectra obtained with vitamin D_3 was similar to that of calciferol under the same conditions, except that the maximum at 625 mμ was lower and shallower, while the maximum at 400 mμ was distinctly higher.

SUMMARY

A spectrophotometric study has been made of a new colorimetric reaction of vitamins D_2 and D_3, given on the addition of glycerol dichlorohydrin or related compounds in the presence of acetyl chloride or other halides of acid nature.

With calciferol an immediate yellow color is formed which turns green in 1 minute, reaches a maximum in 15 minutes, and remains stable at 625 mμ for several hours. With ergosterol an immediate weak pink color is observed which turns to orange in 15 to 20 minutes and later to a fluorescent green. With 7-dehydrocholesterol no color is observed for several minutes; afterwards a faint pink color appears which becomes more intense in 24 hours. With cholesterol no color is produced.

Under conditions suitable for quantitative estimations at 625 mμ, ergosterol has less than 4% and 7-dehydrocholesterol less than 0.3% of the absorption produced by vitamins D_2 and D_3. Cholesterol has no absorption at all.

Conditions were developed for the differentiation of (1) vitamins D_2 and D_3 from ergosterol and 7-dehydrocholesterol, (2) ergosterol from 7-dehydrocholesterol and (3) the dinitrobenzoate esters of the above compounds and the free sterols.

To the authors' knowledge this is the first reaction that distinguishes between ergosterol and 7-dehydrocholesterol.

ACKNOWLEDGMENT

The authors are indebted to Mead Johnson & Company for samples of calciferol and ergosterol; to E. I. du Pont de Nemours & Co., Inc., for samples of 7-dehydrocholesterol and the 3,5-dinitrobenzoate esters of 7-dehydrocholesterol, vitamin D_2, and vitamin D_3; to Winthrop Chemical Company for samples of vitamin D_2 and vitamin D_3; and to Ayerst, McKenna, and Harrison, Ltd., for samples of vitamin D_3.

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Presented in part before the Division of Biological Chemistry at the 107th Meeting of the American Chemical Society, Cleveland, Ohio. This investigation was aided by United Hospital Fund of Greater New York City.

Tests for Aromatic Hydrocarbons

Several proposed testing procedures and specifications for industrial aromatic hydrocarbons have been developed by A.S.T.M. Committee D16, organized in 1944. Subcommittees have carried out work on test methods for crude and refined aromatic products and specifications for aromatic chemicals and solvents.

Methods of Test for Crude Aromatic Products
- Determining refined water-white constituents in light oil

Methods of Test for Refined Aromatic Products
- Scope
- Specific gravity
- Hydrogen sulfide and sulfur dioxide
- Color
- Copper corrosion test
- Distillation (aromatic hydrocarbons)
- Acid wash (benzene, toluene, xylenes, and similar materials)
- Solidifying point (benzene)
- Paraffins
- Acidity

Specifications for Aromatic Chemicals
- Nitration grade benzene
- Industrial grade benzene
- Nitration grade toluene
- Industrial grade toluene
- Nitration grade xylene
- 5° xylene

Specifications for Aromatic Solvents
- Industrial 90 benzene
- 10° xylene
- Industrial xylene
- Refined solvent naphtha
- Crude light solvent naphtha
- Crude heavy solvent naphtha

The committee is eager to stimulate constructive comment, based on practices in current use. Copies of methods and specifications may be obtained from the acting secretary, W. L. Douthett, Texas Co., 135 East 42nd St., New York 17, N. Y. J. M. Weiss is chairman of the committee.

THERMOBAROMETER

Quick and Exact Conversion of Gas Volumes into Weight

ERNST BERL, W. G. BERL, AND G. A. STERBUTZEL
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THE function of the thermobarometer is to assist in the correction of gas volumes obtained in gas analytical determinations to normal temperature and pressure. It includes corrections due to temperature and pressure changes as well as allowing for the vapor pressure of the confining liquid in the gas buret. A known volume of air (100 cc. at NTP in the present instrument) expands or contracts with the continuous changes in atmospheric pressure and temperature of the room in which the gas determinations are carried out. If the air in the thermobarometer and the analysis gas are in equilibrium with the atmospheric pressure and at the same temperature, the volume occupied by the air is substituted in Equation 1 as thermobarometer reading. The necessity for equality in temperature requires that gases evolved in exothermic analytical reactions be allowed sufficient time to return to room temperature. In contrast to the well-known Lunge or Hempel compensator, only one thermobarometer is necessary for an entire battery of gas burets using identical confining liquids. The conversion of gas volume into weight can be carried out with Equations 1 or 2 and Tables I and II.

In the equation:

$$A = \frac{V \times f \times 10}{T.R. \times W} \qquad (1)$$

or

$$\log A = \log V + \log R.F. + \log f + 1 - \log W \qquad (2)$$

A = weight % of constituent Y (Tables I and II) to be determined analytically

V = gas volume measured at room temperature and prevailing atmospheric pressure

f = conversion factor of gas volume into weight of desired constituent (see Tables I and II for a number of the more common conversions)

$T.R.$ = thermobarometer reading at room temperature and prevailing atmospheric pressure

W = weight of sample in grams

$\log R.F.$ = log of reduction factor from Figure 1 for a given thermobarometer reading

The thermobarometer is drawn to scale in Figure 1. It consists of tube A with stopcock 1 with an upper uncalibrated part of 90-cc. volume and the lower calibrated part of 35-cu. cm. volume subdivided into scale divisions of 0.05 cu. cm. and numbered at each cu. cm. Tube B, open to the atmosphere, is placed as near as possible to tube A, having identical though unnumbered calibration marks. Leveling bulb C is connected with heavy rubber tubing to A and B through stopcock 2. Whenever a thermobarometer reading is to be made, the mercury levels in A and B are equalized by opening stopcock 2 with leveling bulb C in position to receive from or add mercury to tubes A and B. When the mercury levels in A and B are identical, stopcock 2 is closed and the volume reading is substituted in Equation 1.

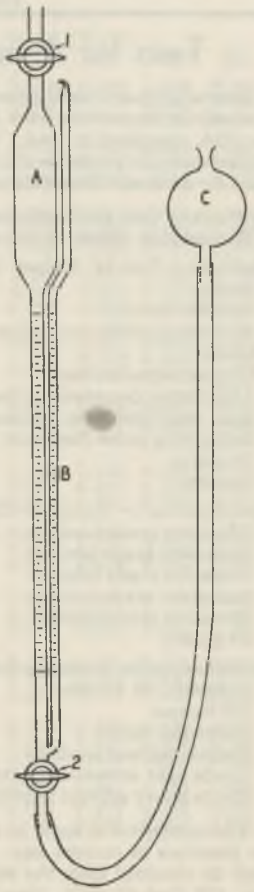

Figure 1

To adjust the thermobarometer initially, temperature and barometric pressure in the laboratory are determined. Assume that a temperature of 25° C. and a barometric pressure of 720 mm. of mercury exist at the time of adjustment. If dry gases—for instance, nitric oxide in the Lunge nitrometer over mercury—

Table I. Conversion Table

Cc. (NTP) of X	Conversion Factor, Mg. of Y into Mg. of Y	Factor	Log Factor
CH_4	CH_4	0.7173	0.8557–1
C_2H_2	C_2H_2	1.172	0.0689
C_2H_2	CaC_2	2.885	0.4601
CO	CO	1.251	0.0972
CO_2	C	0.5396	0.7321–1
CO_2	CH_4	0.7208	0.8578–1
CO_2	CO_2	1.977	0.2961
CO_2	$CaCO_3$	4.496	0.6529
Cl_2	Cl_2	3.220	0.5079
H_2	Al	0.8015	0.9039–1
H_2	Ca	1.787	0.2520
H_2	Fe	2.489	0.3961
H_2	H_2	0.08987	0.9536–2
H_2	K	3.486	0.5423
H_2	Mg	1.084	0.0351
H_2	Na	2.050	0.3118
H_2	Zn	2.915	0.4646
H_2S	H_2S	1.539	0.1873
N_2	$CO(NH_2)_2$[a]	2.956	0.4707
N_2	N_2	1.250	0.0970
N_2	N_2[b]	1.282	0.1080
N_2	NH_3[c]	1.558	0.1927
NH_3	NH_3	0.7711	0.8871–1
O_2	O_2	1.429	0.1550
O_2	Bleaching chlorine in $CaOCl_2$[d]	3.167	0.5006
O_2	H_2O_2[e]	1.518	0.1813
O_2	H_2O_2[f]	3.036	0.4823
SO_2	S	1.465	0.1657
SO_2	SO_2	2.926	0.4663

Factors in Table I are arranged in alphabetical order of the obtained gas and then of the analyzed substances.

[a] Reaction with sodium hypobromite (see [b]). Low yield of 91% due to incomplete reaction is considered in factor.

[b] Ammonium compounds with sodium hypobromite:

$$2NH_3 + 3NaOBr = N_2 + 3NaBr + 3H_2O$$

[c] Ammonia determination in ammonium compounds as in [b].

[d] Bleaching chlorine in bleaching powder with hydrogen peroxide:

$$CaOCl_2 + H_2O_2 = O_2 + CaCl_2 + H_2O$$

[e] Hydrogen peroxide analysis with reaction [d] or with:

$$5H_2O_2 + 2KMnO_4 + 3H_2SO_4 = 5O_2 + 2MnSO_4 + K_2SO_4 + 8H_2O$$

[f] Hydrogen peroxide analysis by catalytic decomposition with Pt, Vd, or Co compounds: $2H_2O_2 = O_2 + 2H_2O$.

Table II. Conversion Table[a]

Cc. of NO (NTP)	Conversion Factor, Mg. of Y into Mg. of Y	Factor	Log Factor
NO	Dinitrotoluene	4.066	0.6092
NO	Glycerol trinitrate	3.380	0.5289
NO	HNO_3	2.814	0.4493
NO	KNO_2	3.800	0.5798
NO	KNO_3	4.515	0.6547
NO	N	0.6256	0.7963–1
NO	NO	1.340	0.1271
NO	NO_2	2.769	0.4423
NO	N_2O_3	1.697	0.2298
NO	N_2O_5	2.412	0.3824
NO	NH_4NO_3	3.575	0.5533
NO	$NaNO_2$	3.082	0.4484
NO	$NaNO_3$	3.796	0.5793
NO	Picric acid	3.410	0.5328
NO	SO_3NH	5.675	0.7540
NO	TNT	3.382	0.5291

[a] Data are in alphabetical order of substance to be analyzed. For other analyses and, for instance, for determination of phenylhydrazine by oxidation with Fehling's solution or determination of nitroso groups with phenylhydrazine or of primary amines with nitrous acid made by measurement of nitrogen—the conversion factors and their logs have to be determined by taking into account the mole weight and the experimental mole volume of the gas developed.

are being measured, 720.0 (pressure scale) in Figure 2 is connected with 25.0 (temperature scale, dry gas) and extended to thermobarometer reading scale. This scale indicates that 100 cc. of dry air at NTP expand to 115.21 at the prevailing pressure and temperature. Air dried with a drying agent, such as phosphorus pentoxide, calcium chloride, etc., is introduced into the dry tube, *A*, which was initially filled with mercury. Stopcock 1 is closed permanently when the mercury in tubes *A* and *B* are level at a scale reading of 115.21.

If the analysis yields gases which are confined over water in the gas buret, the thermobarometer adjustment, for similar temperature and pressure conditions as above, is made by connecting 720.0 (pressure scale) with 25.0 (temperature scale, wet gas) and extending to the thermobarometer reading scale at 119.21. To keep the air in *A* saturated with water at all times, one or two drops of water are introduced through stopcock 1. Water vapor–saturated air is introduced into *A* and the mercury in *A* and *B* is leveled at a scale reading of 119.21.

If analyses are carried out where gases—for instance, carbon dioxide—are collected over saturated sodium chloride solution or, for instance, nitrogen over aqueous potassium hydroxide (1 to 2.5) the reduction of vapor pressure of water, due to the presence of electrolytes, must be taken into account. Two drops of liquid of the same composition as the confining liquid are introduced into *A* before the final adjustment is made. The volume of air to be introduced into *A* is calculated as follows:

Since $v.p._{NaCl_{sat.}} = 0.76\ v.p._{H_2O}$

and $v.p.\ KOH/H_2O\ (1\ to\ 2.5) = 0.65\ v.p.\ H_2O$

the thermobarometer reading for the prevailing temperature (25.0°) and pressure (720.0 mm.) for both dry and wet gas is

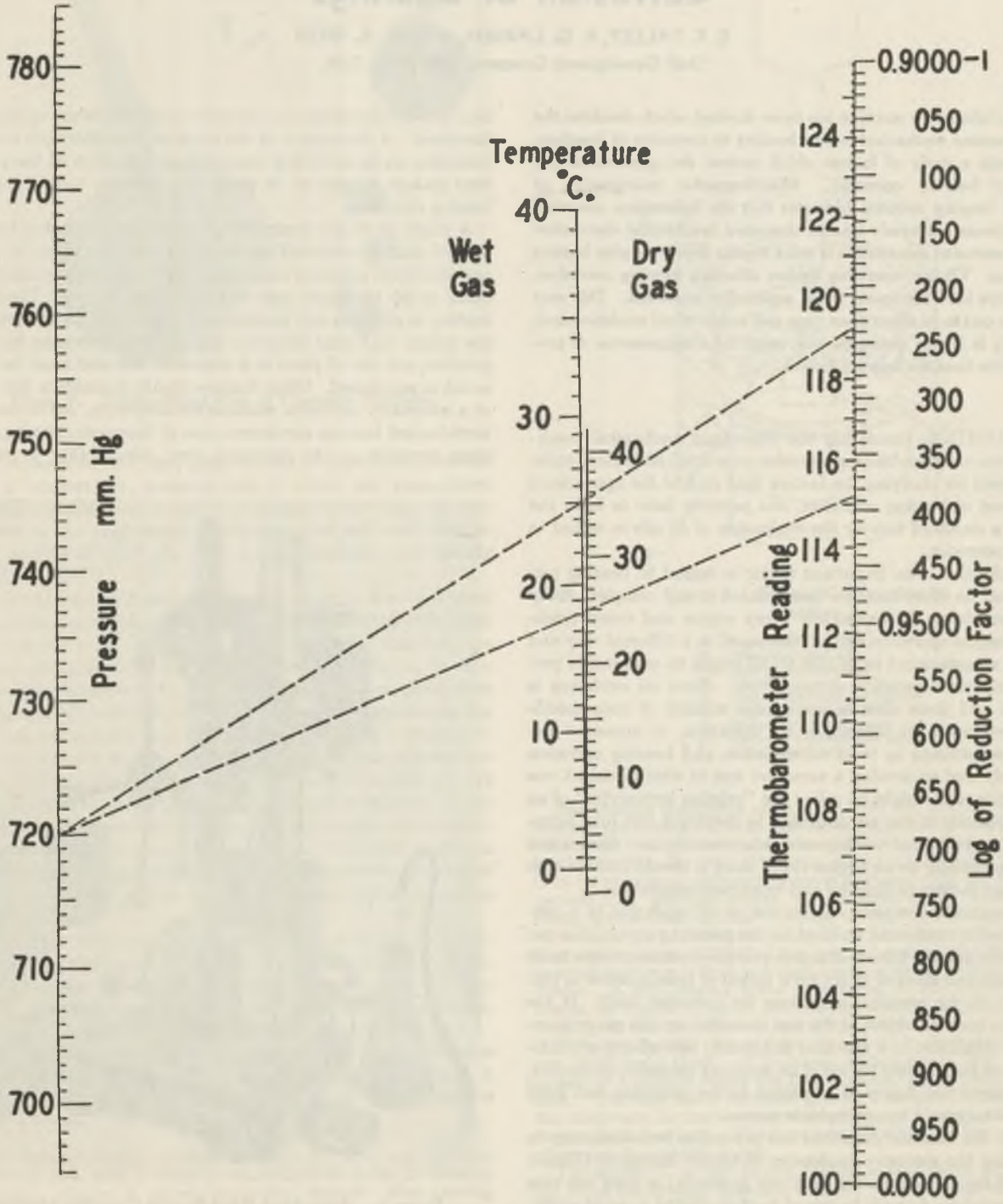

Figure 2. Nomograph

determined and the difference between thermobarometer readings is multiplied by the fractional reduction in water vapor pressure (0.76 for saturated sodium chloride solutions and 0.65 for potassium hydroxide to water 1 to 2.5). This represents the volume occupied by water vapor and is added to the thermobarometer reading based on dry air. Thus for saturated sodium chloride, the volume of air admitted to A is $115.21 + (119.21 - 115.21) \times 0.76 = 118.25$ cu. cm., and for potassium hydroxide (1 to 2.5) $115.21 + (119.21 - 115.21) \times 0.70 = 118.01$ cu. cm. An excess of air which has passed through a washing bottle filled with the confining liquid, say 130 cu. cm., is introduced into A by manipulating stopcock 2 and leveling vessel C, allowed to come into equilibrium with the drops of confining liquid previously introduced, and the excess air expelled by gradual inflow of mercury into A and B. Stopcock 1 is permanently closed when the level of mercury rises to the calculated value.

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A Laboratory Machine for Investigating Corrosion of Bearings

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A simple laboratory machine has been devised which simulates the more important mechanical factors leading to corrosion of bearings, and permits a study of factors which control the appearance and extent of bearing corrosion. Metallographic examination of corroded bearing sections indicates that the laboratory corrosion test specimens are nearly free of unwanted mechanical destruction and that corrosion penetration is more regular than in engine bearing specimens. Of the operating factors affecting bearing corrosion, temperature has been found to be especially important. This may be due in part to its effect upon type and extent of oil oxidation and, especially in highly detergent oils, upon the disappearance of protective films from the bearing surface.

A MACHINE, simulating the important mechanical conditions of engine bearing corrosion on a small laboratory scale, was desired for studying the factors that control the appearance and extent of bearing corrosion, and possibly later to form the basis of a standard test for the evaluation of all oils in regard to bearing corrosion.

Oil oxidation is an important factor in regard to bearing corrosion and its effect must be incorporated in any complete study of the problem. Unfortunately every engine and every condition of engine operation will oxidize an oil in a different way and there is no agreement as to how an oil might be oxidized to provide truly representative deterioration. Since oil oxidation is complex and there already are a large number of tests specifically developed for examining oil oxidation, it appeared desirable to attempt to treat oil oxidation and bearing corrosion separately and to develop a corrosion test in which interest was centered in what might be called the "existing corrosivity" of an oil. Especially it was not intended to develop a test fully simulating the progress of bearing corrosion in an engine. Such a test would essentially be an engine itself, since it should include such important factors as blow-by and crankcase ventilation.

Although the complete elimination of oil oxidation in a corrosion test is considered an ideal for the present purpose, it is not possible to achieve this condition in practice because oxygen must be present and allowed to act for a period of time in order to provide one of the essential conditions for corrosion itself. It has therefore been an object of the test described in this paper to reduce oil oxidation to a practical minimum; the effects of oxidation could be provided by use of an auxiliary oxidation procedure, or if desired the degree of oxidation in the corrosion test itself could be increased by appropriate means.

While the machine described has proved to be satisfactory in predicting the corrosive tendencies of highly detergent (Diesel) oils and for testing used engine oils generally, it does not rate ordinary lubricating oils in regard to their ability to resist oxidation and development of a corrosive condition when operated as described. A description of the machine, together with some information on its operating characteristics, is given at the present time mainly because of its value as a research tool in studying bearing corrosion.

A study of results from current laboratory corrosion tests indicated that an essential condition for correspondence in degree and kind with corrosion experienced in an engine is a high rate of shear in the oil layers next to the bearing surface. Conditions leading to abrasion and to pounding, which may be important to the extent that they intensify the effects of corrosion in actual practice, are out of place in a corrosion test and must be eliminated or minimized. Other features highly desirable in the design of a laboratory corrosion machine are simplicity, use of standard steel-backed bearing specimens, ease of thorough cleaning, minimum demands on the operator's time, adaptability to multiple

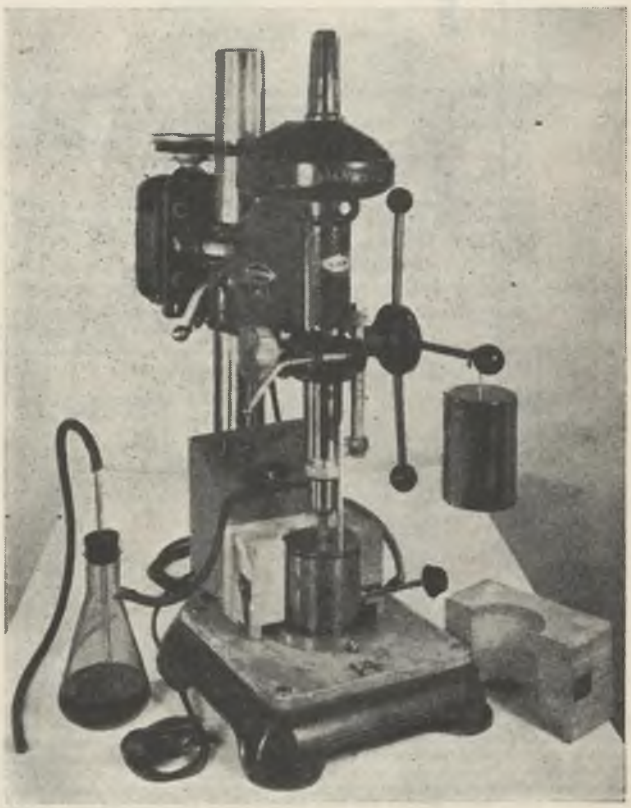

Figure 1. Assembled Thrust Bearing Corrosion Machine

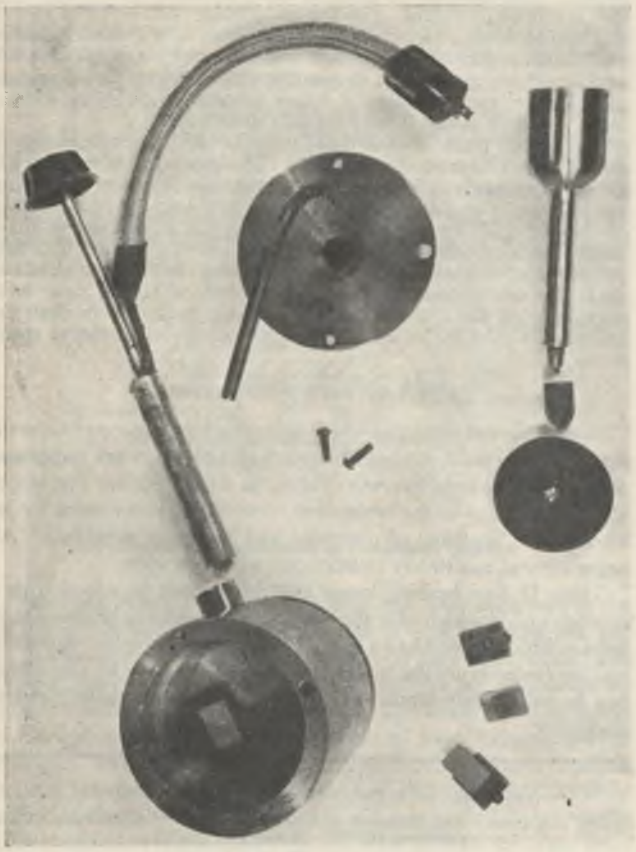

Figure 2. Disassembled Parts of Corrosion Machine

setup, reasonable cost, and good control of experimental conditions of temperature, pressure, rate of shear, and atmosphere. It is also desirable that required oil sample be not large, for convenience in the preparation of experimental oils, and also because samples of used oils from engine crankcases are usually limited in size.

While the various forms of apparatus that have been devised for investigating bearing corrosion have many desirable features, none satisfies all of the above conditions. For example, the Underwood machine (*4*) requires a large oil sample and is difficult to clean; moreover, the shear in the oil layer is not subject to full control. In addition, the method used for accelerating the rate of oil oxidation is highly arbitrary. Test-tube corrosion experiments do not show a correspondence to engine results, apparently because the nearly static conditions under which they are conducted provide little or no shear in the oil layers next to the bearing, and corrosion products, in general, are not carried away. It is probably for this same reason that the Caterpillar corrosion test, in which bearing specimens are rotated at the low rate of 60 r.p.m. in a beaker, fails to rate oils of low corrosivity in the proper order.

Although engine tests must be used as a final criterion of bearing corrosion, they are not satisfactory for development research, because they have insufficient reproducibility and afford no ready means of investigating or controlling the variables under examination; moreover, they require large samples of lubricants.

A machine has been designed to meet the outlined requirements and has been called the thrust bearing corrosion machine. A photograph of the assembled machine with thermostatic control is shown in Figure 1, the parts in Figure 2.

The machine consists of a heavy, cold-rolled steel cup in which a hardened steel disk is made to rotate against three flat bearing specimens. Figure 3 shows important details. The bearing specimens are supported by a single ball-bearing pivot, as shown in Figure 4, and adjust their angle of tilt to give a lubricant wedge and provide essentially hydrodynamic lubrication. This flat type of bearing is called the Kingsbury bearing and has been studied in considerable detail by Muskat and co-workers (*2*).

Cup *A* (Figure 3), which is fitted with a cover, is 5 cm. (2 inches) high inside and has an inside diameter of 6.25 cm. (2.5 inches). Heat is supplied by a 330-watt Chromalox No. A-10 ring unit heater set in a circular recess, *G*, in the bottom of the cup. A Fenwal No. 732RC cartridge thermoswitch, which breaks the full heating current, is placed in a hole, *F*, in the cup between the oil and heater sections. The temperature of the cup is indicated by a thermometer placed in well *I*.

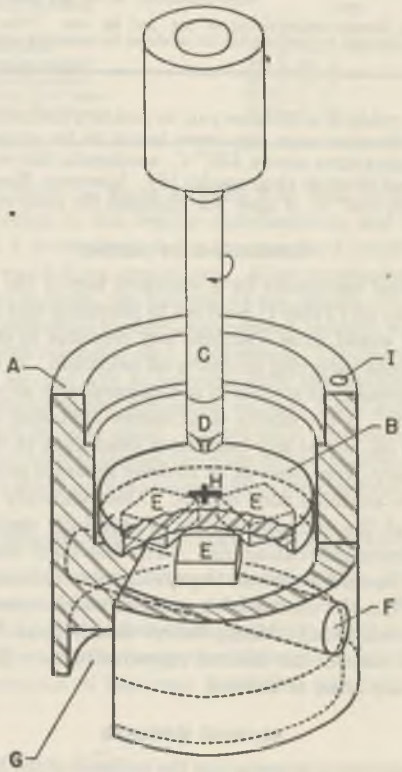

Figure 3. General Construction of Machine

A. Steel cup
B. Hardened steel disk
C. Driving spindle shank
D. Fiber tip
E. Bearing specimen and support
F. Hole for thermoswitch
G. Recess for electric heater
H. Recess to accommodate tip *D*
I. Thermometer well

The bearing specimens are 1.25 × 1.88 cm. (0.5 × 0.75 inch) and are preferably cut from flat stock supplied by bearing metal manufacturers. The regular curved inserts can be flattened and used if a particular type of bearing, not available in the flat form, is to be examined, although there is a danger that the flattening process may produce cracks, as discussed later. Flat bearing stock, copper-lead on steel backing, of very uniform quality can be secured at a reasonable cost from the Cleveland Graphite Bronze Company, 17000 St. Clair Ave., Cleveland 10, Ohio. Each bearing specimen is held in a steel cradle without a rigid clamping device. A ball-bearing pivot is inserted in the bottom of the cradle, by means of which the bearing assumes a definite tilt according to the conditions of speed and pressure.

The steel bearing disk is 5 cm. (2 inches) in diameter by 0.625 cm. (0.25 inch) thick. It is driven with a bench drill press by means of a spindle which fits into a recess, *H*, in the top of the disk. The disk has a hardness greater than 300 Brinell; this is the minimum hardness recommended for use in conjunction with copper-lead bearings. The hardness requirement for cadmium-silver and for babbitt is less.

The shank, *C*, of the driving spindle is cold-rolled steel. The tip, *D*, of the driving spindle is of fiber; this material was chosen because a steel tip would wear against the disk and produce suf-

Table I. Standard Conditions for Operation of Thrust Bearing Corrosion Machine

Duration of run	20 hours
Temperature	225° F. (107° C.)
Oil charge	35 cc.
Thrust	Approximately 125 pounds
Weight on drill press arm	5 kg.
Speed	2400 r.p.m.
Bearing specimens	New Cu-Pb
Bearing specimen surface	Polished (No. 0000 Hubert), slightly beveled edges
Bearing specimen thickness	All three specimens should agree in thickness to within 0.002 inch
Bearing specimen source	Cleveland Graphite Bronze Company
Steel disk hardness	300 Brinell (minimum)
Steel disk surface	Mirror finish
Air rate	Approximately 30 cc. per minute

All bearing losses are reported as mg. per sq. cm. Prior to weighings, bearings are cleaned by boiling for 10 minutes in naphtha and in acetone.

ficient iron oxide in a 20-hour run to create a distinct turbidity in the oil, while aluminum tips were found to be subject to breakage. Temperatures above 130° C. accelerate the wearing of the fiber tips and shorten their useful life; however, fiber tips can be used even at 180° C. if they are changed for each run.

CONDITIONS OF TESTING

The general conditions for a standard test of the existing corrosivity of an oil (Table I) were set to provide a test of reasonable length that would show a satisfactory response to corrosive conditions without incurring excessive oil oxidation. It was intended that corrosiveness be measured as independently as possible of all other factors; if the effects of extensive oxidation on corrosiveness of the oil, under any particular conditions in the engine or the laboratory, were desired, an entirely separate procedure could be added to examine such effects. This relatively independent treatment of the oxidation factor is especially useful for detergent-containing lubricating oils. (The test itself could of course be readily changed if desired to provide more extensive oxidation of the oil than the unavoidable amount which occurs under the standard conditions.) Manipulative details have been fixed as required to secure the desired reproducibility. The standard conditions are listed in Table I.

BEARING SURFACES

The requirements in preparing the surfaces of the bearing specimens and the steel disk were investigated with both corrosive and noncorrosive oils. Comparatively rough surfaces of the steel disk were used in the tests with the corrosive oils and the greatest bearing loss was obtained with the roughest disk, the bearing losses ranging between 30 and 60 mg. per sq. cm. With a noncorrosive oil and polished copper-lead bearing specimens, a mirror finish of the steel disk gave slightly lower average losses than a semimirror finish, 0.09 mg. per sq. cm. as compared with 0.17 mg. per sq. cm. This slight difference approached the probable reproducibility of the test. It was concluded that while a semimirror finish is entirely suitable for most work, and a mirror-finished disk is an essential requirement only when testing small differences of slightly corrosive oils, the latter is sufficiently easily prepared to warrant selecting as standard.

No significant change in the bearing losses is produced by substituting a smooth, dull lapped finish on the copper-lead bearing specimens for a fully polished finish. Even the comparatively rough surface of unfinished specimens, taken directly from an intermediate step in the process of bearing manufacture, does not increase the bearing loss, provided that the load is supported over the whole bearing surface. While the condition of the bearing surface is thus not critical, a polished surface is easily obtained and assures elimination of a possible source of variation in results from this cause.

The steel disk and the bearing specimens are surfaced by the usual metallographic methods. The bearing specimens are finished on No. 0 and No. 0000 Hubert papers. The steel disk is carried to a semimirror finish by hand on No. 400 "Speed-Wet" paper. The final mirror finish is made mechanically. The steel disk is placed off center on an easily-turning, cloth-covered 15-cm. (6-inch) disk that replaces the thrust bearing corrosion machine in the drill press. The steel disk is driven with its regular spindle and its off-center position causes the cloth-covered disk to rotate at a slower speed. Using a water suspension of levigated alumina, a mirror polish is very quickly obtained.

A trial was made of steel disks superfinished through the kindness of the Chrysler Corporation. The Superfinish process (*3*) gives an exceptionally smooth and reproducible surface with a minimum of metal distortion. Using copper-lead bearing polished on No. 0000 Hubert paper, two runs with superfinished disks using a noncorrosive oil gave bearing losses of 0.04 and 0.07 mg. per sq. cm. These values were comparable with those obtained with the mirror-finished disks. However, after the runs with superfinished disks, there was a noticeable reduction in both the extent and number of scratches in the disks and bearing specimens.

EFFECT OF SPEED AND PRESSURE

It is believed that, as long as hydrodynamic conditions of lubrication prevail, changes in speed and pressure are important only as they change the rate of shear in the oil layer. The major effect of each, as so far recognized, is to increase corrosion by removing the products of corrosion and replacing reactants. All experimental results are in harmony with this view.

Table II lists bearing losses that have been obtained with a corrosive oil in which only the conditions of speed or pressure have been altered. Speed has a greater effect than pressure; however, neither factor is critical under the conditions of the test and both can be held within limits sufficiently close for satisfactory reproducibility.

Table II. Effect of Varying Speed and Pressure

[Fixed conditions: Oil, reference oil 1 (corrosive). Time, 20 hours. Temperature, 107° C. Bearings, Cu-Pb]

Thrust	Bearing Losses			
	610 r.p.m.	1230 r.p.m.	2400 r.p.m.	3600 r.p.m.
Lb./sq. in.	*Mg. per sq. cm.*			
15	...	..	22.2 23.2	29.7
125	5.2 4.2	16.7 20.3	28.7 29.6	

VISCOSITY OF LUBRICANT

In establishing a bearing corrosion test, it is important to know whether the conditions of speed and pressure are too severe for the least viscous oils that will be tested. There is a possibility that with the less viscous oils, hydrodynamic lubrication may fail and bearing losses will result from metal to metal contact as well as from corrosion. It is not possible to investigate bearing corrosion in respect to viscosity alone as an isolated variable unless conditions are so mild that oxidation is not important. This restriction arises because oils of different viscosities, even from the same base stock, have different chemical characteristics. The important point is whether, under conditions in which corrosion is absent, there is a difference in behavior of oils of different viscosity in the test.

A series of runs was made on various grades of commercial motor oils in both the 50 and 100 VI series. The losses of copper-lead bearings were less than 0.3 mg. per sq. cm. in all cases and, within these limits, were not significantly greater for the S.A.E. 10 and 20 grades than for the more viscous oils. It is concluded that, within the viscosity range of commercial lubricating oils, viscosity is not a factor in bearing corrosion as measured in the thrust bearing corrosion machine.

GENERAL TECHNIQUE

Passivation of the machine was investigated by comparing different cleaning methods, by using a new machine, and by adding iron wire. The tests were made with a mildly corrosive oil, reference oil 2 (corrosive), which was highly sensitive to conditions of the machine affecting oxidation. When, as usual, the machine

was cleaned between runs by washing in naphtha and acetone, the bearing corrosion loss with reference oil 2 was 0.3 mg. per sq. cm. No change occurred when the machine was further cleaned by soaking in dilute hydrochloric acid until bubbles of hydrogen appeared over the entire cleaned surface. No change was produced in another run in which the machine was cleaned by boiling successively in isopropyl alcohol containing 10% potassium hydroxide, then in isopropyl alcohol followed by several changes of water. In a third run, a new machine body was used while the bearing supports, spindle, and disk were given the alcoholic potassium hydroxide wash just described; results were the same.

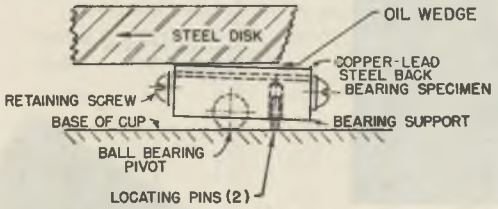

Figure 4. Formation of Lubricant Wedge

Two runs were made with analytical iron wire immersed in the oil during the test. The wire was used in ratio of 3.5 sq. cm. per ml. The corrosion rates with reference oil 2 were increased from 0.3 mg. per sq. cm. to 1.4 and 4.2 mg. per sq. cm., showing that an extended iron surface can accelerate the oxidation of the oil in the test and change the oil from noncorrosive to definitely corrosive. The total area of iron immersed in oil in the machine is 150 sq. cm. The run with the new machine body indicated that a fresh iron surface is not particularly catalytically active, and the experiments with iron wire indicate an upper limit of bearing loss that can be expected from this source. These experiments do not show the effect of a truly clean iron surface; they only show ordinary cleaning methods give reproducible results.

To maintain a uniform atmosphere it is the practice to pass air from the compressed air lines through the machine during a run. The compressed air is dehydrated at ambient temperatures and at pressures ranging from 4.2 to 7.0 kg. per sq. cm. (60 to 100 pounds per sq. inch) by draining off the condensed moisture, and thus the partial pressure of water vapor of the air as used at one atmosphere is from 2 to 8 mm. of mercury. A run was made with reference oil 2 (corrosive) in which the partial pressure of water vapor was increased to 25 to 30 mm. of mercury; the corrosion loss was increased to 1.2 mg. per sq. cm. Thus water vapor can cause a change in the measured bearing corrosion and it should be held to a relatively constant value.

Using reference oil 1 (corrosive), the importance of equality of height of the bearing surfaces was examined by making several runs with one bearing specimen 0.10 mm. (0.004 inch) thicker than the other two. The results of the tests are shown in Table III. The same correspondence that is obtained between the three bearing specimens in other runs, in which the bearing specimens were matched in thickness to within 0.03 mm. (0.001 inch), was obtained in the present series.

Table III. Effect of Nonuniformity of Bearing Specimen Thickness
(Corrosive reference oil 1)

Loss in Weight		
Specimen 1	Specimen 2	Specimen 3 (0.004 Inch Excess Thickness)
Mg.	*Mg.*	*Mg.*
65.4	68.3	64.1
69.3	77.2	75.6
81.7	85.5	83.5

Table IV. Corrosion Losses of Bearing Metals
(Corrosive reference oil 1)

Type of Bearing	Loss
	Mg./sq. cm.
Copper-lead	28.2, 28.7, 25.9, 28.9
Cadmium-nickel	30.3, 36.5
Cadmium-silver	31.1, 40.0
Babbitt, lead base	0.12, 0.41
Babbitt, tin base	0.20, 0.28

BEARING METALS

A number of steel-backed bearing metals, representative of the more important commercial types used in internal combustion engines, have been tested for corrosion resistance in the thrust bearing corrosion machine using reference oil 1 (corrosive). The results are shown in Table IV. The babbitts show a relatively high resistance to corrosion while the harder bearing metals, copper-lead, copper-nickel, and cadmium-silver, are all subject to corrosion.

A new sandwich type of bearing, recently introduced, consists of a sintered layer of copper and nickel on a steel back with a thin protective layer of babbitt on the bearing surface. A sample of flat bearing stock was secured from an intermediate stage of manufacture, resurfaced to leave at least 0.05 mm. (0.002 inch) of babbitt according to the regular specifications, and tested with reference oil 1 (corrosive) under the standard conditions. The bearing loss was 0.2 mg. per sq. cm. After resurfacing to remove the protective babbitt, the loss was 44 mg. per sq. cm. For comparison, a commercial formed replacement bearing was flattened and tested with reference oil 1 (corrosive). The bearing loss was 2.0 mg. per sq. cm. It is believed that the flattening process opened up cracks in the protective babbitt layer and permitted some corrosion to take place. Errors may, therefore, vitiate results obtained with flattened bearings of the sandwich type and the use of flat stock only is recommended for this type.

SURFACE IRREGULARITIES, FRICTION, AND TEMPERATURE RISE

An unexpected and sometimes uncontrollable generation of heat has been encountered in occasional runs. Because the quantity of heat was entirely out of keeping with the frictional heat to be expected in hydrodynamic lubrication and because the unusual generation of heat was associated with either dirty or

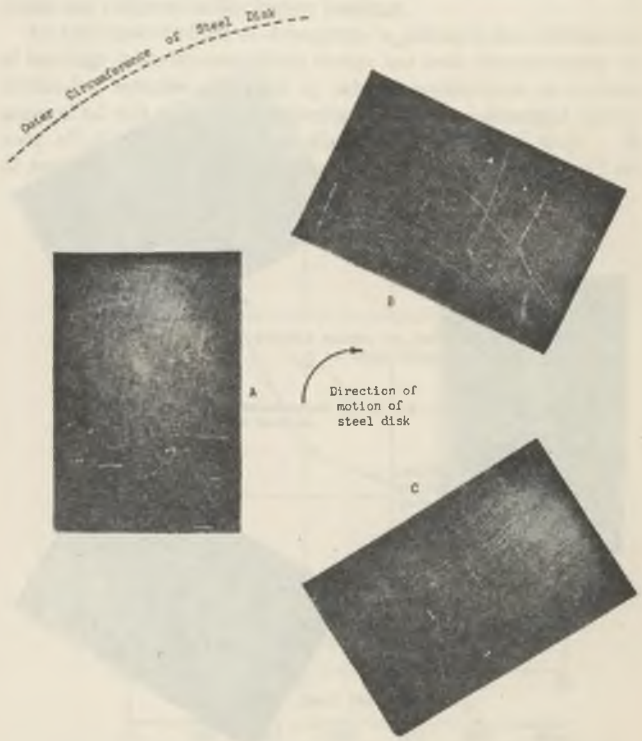

Figure 5. Normal Appearance of Copper-Lead Bearings
After thrust bearing corrosion test with clean noncorrosive oil

corrosive oils, the phenomenon has been studied in some detail through microscopic examination of the bearing specimens, and through measurements of the coefficient of friction and of temperature rise on the bearing surfaces.

Used bearing specimens from the tests showed differences, evident even without the microscope, between bearing specimens from runs involving unusual frictional heat and those from normal runs. The latter have a smoother and more regular appearance. An example of the appearance of used copper-lead specimens, at magnification of approximately ×2, from a run with a clean, noncorrosive oil is shown in Figure 5. The circular scratch marks are superficial and characteristic of most used bearing specimens. Such marks appear on bearings that show no appreciable loss of weight. The relatively large brighter patches on the trailing ends are indicative of thinner lubricating oil films in this area and give assurance that the primary requirement of hydrodynamic lubrication, the setting up of a lubricant wedge, has been attained in the design of the machine.

Figures 6 and 7 illustrate the marked difference in bearing specimens obtained in runs that showed unusually high frictional heat. Examination under the microscope showed that the characteristic feature of these used bearing specimens is the presence of small raised areas. Two types may be distinguished. Thus the bearing specimens from a run in which an undoped mineral oil containing 2% suspended asphaltenes was used are shown in Figure 6. (The asphaltenes were suspended by ultrasonic vibrations.) The raised areas are compressed smears of glossy black asphaltenes and appear as black patches in the photograph. This run was stopped after about 4 hours because of excessive frictional heat. The other type of raised area is exemplified by Figure 7, showing bearing specimens from a run with a corrosive oil in which excessive heating from friction occurred. The raised areas are corrosion residuals. They appear in the figure as polished metallic plateaus; their color is that of burnished copper.

The height of the small raised areas on the used bearing specimens was measured with a microscope at a magnification of ×240. The accuracy of the measurement of the relative heights of two parts of the bearing surface in one field of view was limited to

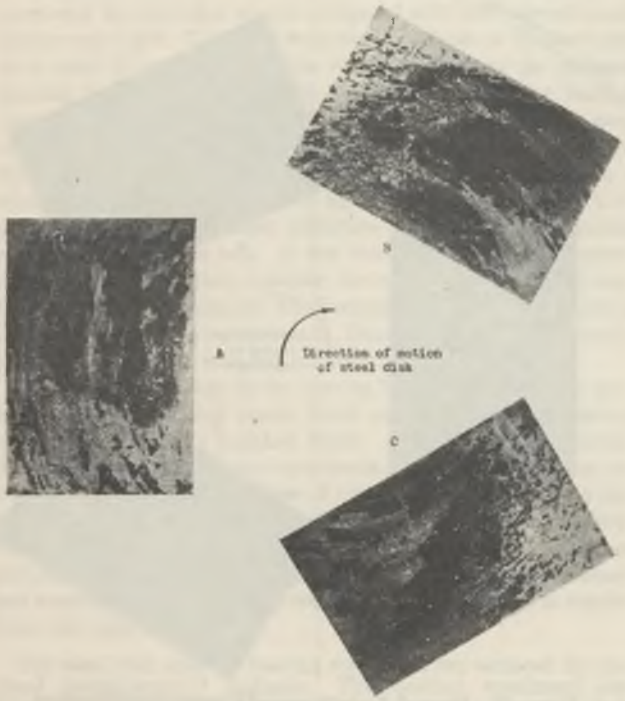

Figure 6. Appearance of Copper-Lead Bearings after Test with Noncorrosive Oil Containing Sludge

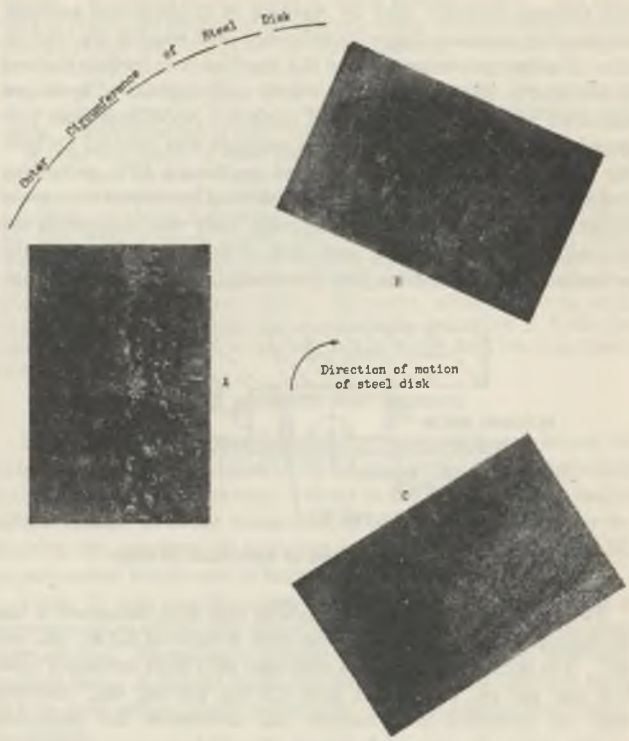

Figure 7. Appearance of Corroded Copper-Lead Bearings after Test with Corrosive Oil

Reference oil 1 used. Note development of corrosion residuals which appear on light areas in A and to a lesser extent in B

0.002 mm. by the sharpness of the focus. Table V lists the average elevation of the raised areas of at least nine fields of view on each bearing specimen examined.

The newly polished unused specimens seldom show areas in any single field that have elevations greater than the probable error of measurement. All used specimens show development of raised areas on the bearing surface. In runs where no unusual frictional heat was developed, the average elevation of the raised areas was from 0.003 to 0.006 mm., while in runs showing high frictional heat, the average elevation was several times as great.

The coefficient of friction was measured directly by means of a dynamometer attached to an altered thrust bearing corrosion machine. The body of the machine was mounted directly on a thrust ball bearing and was free to rotate through a small arc. A thread, attached to the periphery of the body, transmitted the force resulting from friction to a spring dynamometer with a direct reading scale. The air tube and electrical connection to the machine were removed for the short times necessary to measure the frictional force.

The coefficient of friction for most oils proved to range between 0.004 to 0.009. When the coefficient of friction exceeded 0.010 for an hour or more, the machine overheated. However, increases of short duration generally caused no harm.

TEMPERATURES OF SURFACE

Surface temperatures were determined by means of thermocouples imbedded in the surface of the copper-lead bearing specimens.

The leads were brought out between the bearing support and the specimen, in grooves cut into the steel backing. Use of No. 36 B. & S. copper and constantan single silk covered wires was satisfactory even when six leads were placed in one bearing. In making the thermocouple connections to the bearing, a No. 28 drill hole was bored through the steel and about halfway through the copper-lead layer. A sharp needle was used to pierce the copper-lead completely. The two thermocouple wires were

Table V. Elevation of Raised Areas on Used Copper-Lead Bearing Specimens

Oil	Average Elevation	Maximum Elevation of Any One Area
	Mm.	*Mm.*
None (unused bearings)	0.001	0.004
Mineral oil	0.003	0.009
Same plus 2% added asphaltenes ex Indiana test	0.018	0.023
Reference oil 1 (corrosive)	0.010	0.016

Table VI. Effect of Temperature on Corrosion

(Corrosion losses, in mg. per sq. cm. 20-hour run, 125-lb. thrust, 2400 r.p.m.)

Oil	Bearing	100° C.	107° C.	110° C.	120° C.	130° C.	140° C.	150° C.	160° C.	170° C.
A	Cu-Pb	...	0.3	...	...	0.2	0.0	−0.3	...	−0.2
		...	0.4	...	...	0.3	...	−0.2	...	...
B	Cu-Pb	...	0.4	0.1	0.6	0.1	33.5	44.7	42.8	...
		...	...	...	...	0.1	42.6	...	...	...
		...	...	...	...	0.2	...	...	...	...
		...	...	...	...	0.0	...	...	...	...
C	Cu-Pb	0.1	0.1	0.1	11.1	...	...	...	...	...
		...	0.2	...	12.8	...	...	...	...	...
C	Cd-Ag	0.0	...	0.0	0.0	47.0	105.8	...	...	...
		...	...	...	0.0	45.5	55.3	...	...	...
D	Cu-Pb	...	0.2	...	0.1	1.3	9.6	9.2	8.0	...
		...	0.1	...	0.2	0.5	...	10.5	6.6	...
D	Cd-Ag	...	...	...	0.0	0.1	5.4	0.3	...	...
E	Cu-Pb	0.2	0.6	...	20.6	33.9	...	...	...	...
		...	0.5	...	21.6	...	...	...	...	...
		...	1.4	...	14.8	...	...	...	...	...
		...	0.3	...	...	...	...	...	...	...
F	Cu-Pb	...	...	...	...	...	0.1	1.4	24.2	23.9
		...	...	...	...	...	...	6.1	...	...
G	Cu-Pb	...	...	...	0.1	1.4	13.5	...	...	...
H	Cu-Pb	...	...	...	...	0.0	0.1	0.1	0.1	...
I	Cu-Pb	...	...	...	0.2	1.4	0.9	1.2	0.7	...
		...	...	...	...	3.0	1.2	0.7	...	...

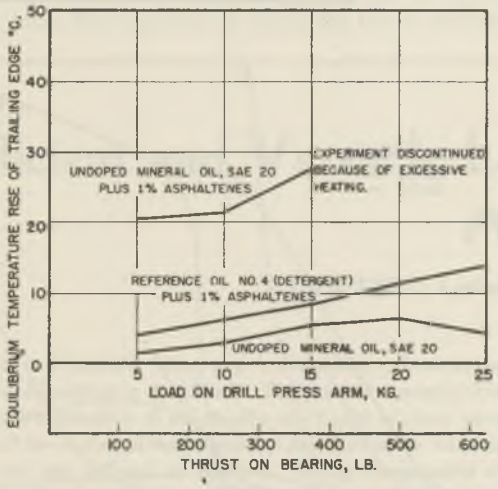

Figure 8. Effect of Asphaltenes and Detergents upon Bearing Temperature Rise

inserted in the hole from the back and pulled through as far as the insulation would allow, soft-soldered on the front side, and the excess clipped off flush with the bearing face. The bearing specimen was then surface-finished according to the usual methods. In some of the later experiments, a direct reading for the increase in bearing temperature over the oil temperature was obtained by placing the second or reference thermocouple in the oil.

In order to discover if the method of inserting the thermocouple in the bearing gave a false temperature by interfering with the regular processes of heat transfer, comparative temperature measurements were made with and without a piece of paper inserted between the bearing specimen and the support. No difference was found.

The temperature of the surface of the bearing specimen was greatest at the trailing edge. In the following discussion the temperature rise of the surface of the bearing at the trailing edge over the temperature of the main body of oil will be designated by ΔT and the coefficient of friction by f.

The nominally recorded gross temperature of the machine agrees with the temperature of the main body of oil within 4° C. under conditions of the standard thrust of 125 pounds per sq. inch. Under the same conditions, ΔT does not exceed 6° C. and the nominal temperatures are thus within 10° C. of the highest bearing temperatures. This relationship applies only when unusual conditions are absent and f is less than 0.010.

The variation of ΔT and f with bearing pressure was investigated up to a thrust of 625 pounds per sq. in. The initial values of ΔT and f, immediately after applying a new, higher load, were always greater than the values after equilibrium had been established. The greatest differences were usually with the higher loadings but in all cases substantial equilibrium was established in 5 to 10 minutes. The most probable cause of the delay was a slowness of the bearing support in changing its tilt to suit the new conditions of loading, perhaps due to friction against the two restraining pins that locate each bearing support. However, trials of other locations of the supporting pivot closer to the trailing edge and of other designs of the support (to eliminate the two pins) produced no improvement in reducing the time to reach a steady frictional state. Direct measurement of the restraining forces could not be made. Since the machine is operated under steady conditions only, attention has been restricted to the values of ΔT and f after steady conditions have been obtained.

The total energy converted to heat is almost proportional to the thrust, since the coefficient of friction decreases only slightly as the load on the bearings is increased. Overheating of the machine was frequent at the higher loadings.

An anticipated effect of detergents in aiding in the lubrication of bearings in an oil containing sludge has been demonstrated by examining samples prepared by adding asphaltenes to a plain mineral oil and to an oil containing an effective detergent (reference oil 4). Figure 8 shows that the addition of only 1 % of asphaltenes, from the Indiana test, to a plain mineral oil re-

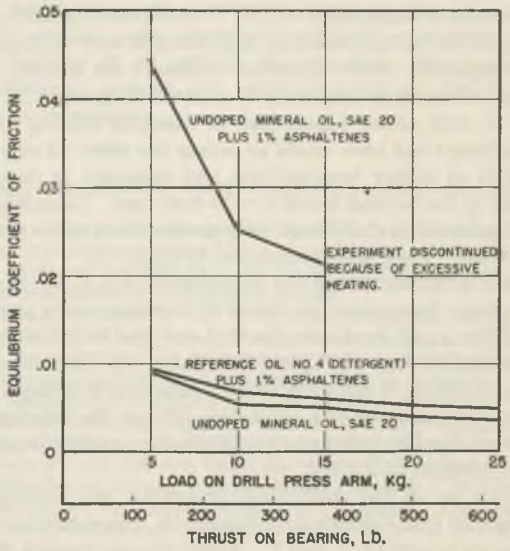

Figure 9. Effect of Asphaltenes and Detergents upon Coefficient of Friction

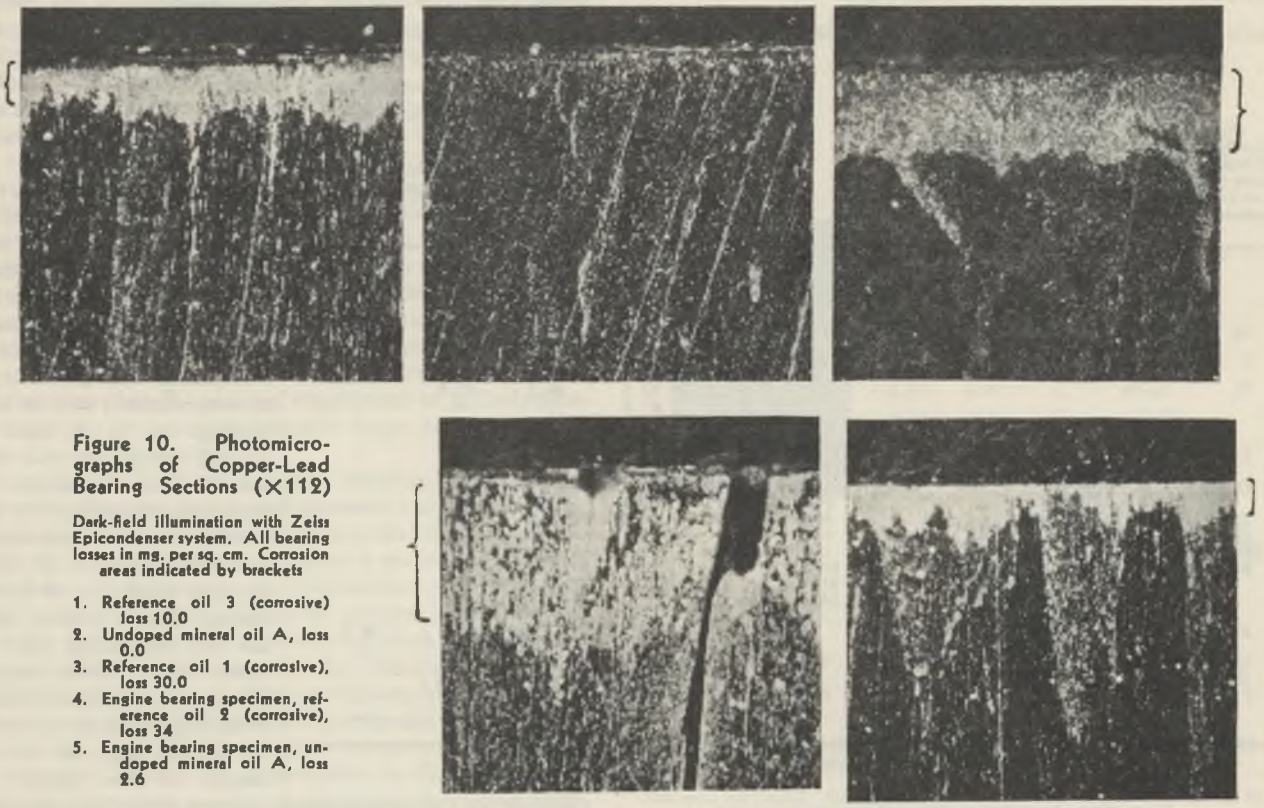

Figure 10. Photomicrographs of Copper-Lead Bearing Sections (×112)

Dark-field illumination with Zeiss Epicondenser system. All bearing losses in mg. per sq. cm. Corrosion areas indicated by brackets

1. Reference oil 3 (corrosive) loss 10.0
2. Undoped mineral oil A, loss 0.0
3. Reference oil 1 (corrosive), loss 30.0
4. Engine bearing specimen, reference oil 2 (corrosive), loss 34
5. Engine bearing specimen, undoped mineral oil A, loss 2.6

sults in a large increase in ΔT at the standard thrust of 125 pounds per sq. in. and the effect increases at higher loadings. The experiment had to be discontinued before the thrust could be increased to 500 pounds per sq. inch because the temperature was out of control and rose well above 200° C. Figure 9 shows a parallel effect upon f. When the same quantity of asphaltenes was added to reference oil 4, the change in f was small and the change in ΔT became pronounced only at the higher thrusts.

EFFECT OF TEMPERATURE

The thrust bearing corrosion machine has proved to be a highly useful tool when the corrosivity of oils is measured at different temperatures and considerable effort has been spent in examining the effect of this variable. While in most cases a 20-hour test at the standard temperature of 107° C. involves only mild oxidation of an oil, higher temperatures may result in very extensive oxidation, especially when oxidation inhibitors are absent. For example, white oil is converted to a semisolid mass in 20 hours at 180° C. with either copper-lead or cast-iron bearing specimens. No attempt has been made to isolate the effect of oil oxidation as such at higher temperatures and attention is directed primarily to the bearing losses in a 20-hour test. Table VI contains bearing loss data at different temperatures for a series of representative oils. These data are plotted in Figure 11.

Oil A is representative of a noncorrosive S.A.E. 30 western oil.

A sharp dependence of extent of corrosion upon temperature has been noted for those oils that are characterized chiefly by detergent activity—B, C, and E in Table VI. It is believed that the appearance of corrosive activity is mainly dependent upon the presence or absence of a protective film on the bearing surface, and that the film is lost at a particular temperature depending on the conditions of the test.

When an oil has antioxidant properties, the development of corrosivity is less abrupt with increase in temperature of the test, as shown by oils D, F, and I. These oils also contain detergents and may be contrasted with B, C, and E discussed above; apparently in the present case the corrosiveness depends mainly on

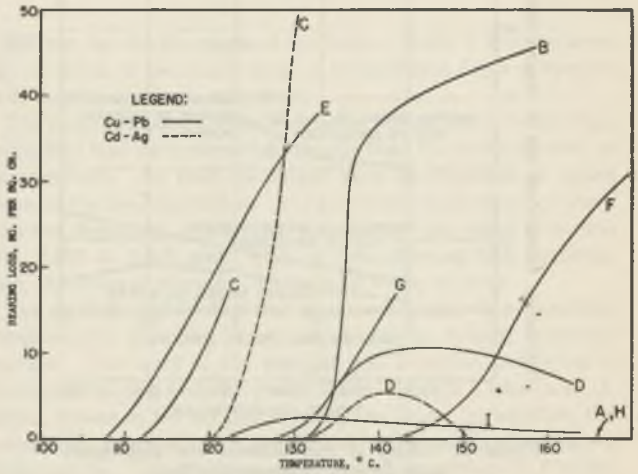

Figure 11. Effect of Temperature on Corrosion

Summary of 20-hour tests on oils A, B, C, D, E, F, G, H, and I

the eventual development of acidity through oxidation of the oil. Oil H is representative of oils that contain corrosion suppressors.

METALLOGRAPHIC EXAMINATION OF BEARING SPECIMENS

Metallographic examination of bearing sections perpendicular to the bearing surface, prepared as described by Grange (*1*), gives information on the nature of bearing corrosion. Examples of representative sections are shown in Figure 10. The penetration of corrosion is evident as a light-colored horizontal band, set off in the figure by brackets, which increases in depth as the corrosion loss values increase. Visual inspection under the microscope shows this light band as a brighter, richer copper color than the copper color of the unaltered copper-lead matrix. Assuming that the original surface is preserved and only lead is lost, comparison of the values of bearing loss per square centimeter with the meas-

ured depth of corrosion penetration indicates that from one half to two thirds of the available lead is lost. This figure is only approximate and may be expected to vary greatly in different specimens.

The penetration of corrosion is generally regular in the thrust bearing corrosion machine bearing specimens and comparatively irregular in engine specimens. The latter show numerous areas where the residual metal structure has broken down and cavities appear in the metal section. The metal structure is sometimes so weakened in spots that any save the most gentle polishing would show such areas as fully developed cavities. The differences in uniformity of penetration in the laboratory and engine specimens can be seen in the photomicrographs but are more evident by visual inspection of larger fields.

Thus in comparison with engine specimens the thrust bearing corrosion machine specimens have the advantage of greater freedom from mechanical breakdown, and at the same time show comparable sensitivity to corrosive action. The disintegration of a bearing resulting from the loss of one constituent or from mechanical pounding is a secondary action and need not be considered if the primary action of corrosion itself can be measured.

EVALUATION OF RESULTS OBTAINED IN THRUST BEARING CORROSION MACHINE

From the information on oils on which service records are available, general indications are that any oil showing a corrosion loss in a 20-hour thrust bearing corrosion test greater than 1 mg. per sq. cm. should be regarded as potentially corrosive and those showing a corrosion loss of 5 mg. per sq. cm. or more, very definitely corrosive. Losses 0.3 mg. per sq. cm. or less are considered negligible under the conditions in which the test is carried out. However, since in actual long-time operation, especially under severe or unusual conditions, some oils in this latter class may also become corrosive, it is not safe to assume noncorrosivity from the test without special examination of the oxidation characteristics of the sample.

The temperature has been shown to be very important in respect to the development of corrosiveness and should be considered in carrying the results of the laboratory test into practice. Oils which are corrosive below 100° C. can be expected to give undue corrosion in engines.

While the reproducibility of the thrust bearing corrosion test is good, it is desirable to make two tests to decide any particularly important point. When the corrosivity changes abruptly with temperature, the reproducibility in respect to the corrosion loss near the critical temperature is poor because small differences in conditions make large differences in the result.

The authors would be glad to furnish blueprints to those who may desire to construct this machine.

ACKNOWLEDGMENT

The authors wish to acknowledge the able assistance of Leo Grenot in the experimental work.

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PRESENTED before the Division of Petroleum Chemistry at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J. Publication delayed at the request of the Office of Censorship.

Efficient and Versatile Laboratory Fractionation Column Assembly

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An efficient and versatile laboratory fractionation column assembly with improved features is described. The column assembly can be used for the fractionation of practically any liquid mixture normally encountered in analytical or research laboratories, in volumes as small as 2 cc. and as large as 5000 cc., having components which boil from room temperature to 400° C. (752° F.), with a fractionation efficiency equivalent to 30 to 50 theoretical plates. The fractionation efficiencies and large throughput capacities of the packings enable complex mixtures to be separated in a minimum time. Mixtures with components whose boiling point differences are as little as 5.9° C. can be readily fractionated. The newly developed staggered dual heating unit enables a packed or plate laboratory column to operate under essentially adiabatic conditions with complete visibility to all parts of the apparatus. By means of specially designed supports, the entire apparatus can be rapidly assembled or dismantled.

AN EFFICIENT and versatile laboratory fractionation column assembly has been developed and used extensively by the author in his research projects of analytical and synthetic organic chemistry. Several improvements are incorporated in this fractionation column assembly which not only greatly extend its range of application with improved fractionation efficiency but also make it a generally useful piece of laboratory equipment.

The newly developed staggered dual heating unit enables any type of packing or plate column to be readily operated under essentially ideal adiabatic conditions at temperatures ranging from room temperature to as high as 400° C. (752° F.) for atmospheric or vacuum fractionations. This heating unit not only permits complete visibility to all parts of the apparatus during fractionation but also eliminates the use of fragile and expensive silvered vacuum jackets.

By means of three interchangeable fractionation columns, it is possible to fractionate volumes from 2 to 5000 cc. with a fractionation efficiency equivalent to 30 to 50 (H.E.T.P. 1.8 to 3.0 cm.) theoretical plates in a minimum of time.

A still head, common to the three interchangeable columns, has a total holdup of less than 0.5 cc. under operating conditions for any of the columns. Its compactness enables it to be placed within the heated jacket, and thus greatly aids in extending the operating temperature range of the fractionation assembly.

The micro control reflux regulator was used for its exceptionally low holdup of 0.2 cc., simplicity, compactness, accuracy, stability of control, and extreme range for controlling reflux ratios. It can readily be set for reflux ratios ranging from 1–1 to 100–1, for total reflux, or for total collection of distillate. The reflux regulator has a throughput capacity ranging from 0.2 to 50 cc. per minute.

An improved spiral packing for small-diameter columns is also described which has been found by the author to be the most effi-

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cient of the many tested. Mixtures of *o*- and *p*-xylenes, boiling 5.9° C. apart, can be readily fractionated. This packing has a fractionation efficiency equivalent to 50 (H.E.T.P. 1.8 cm.) theoretical plates, as well as a relatively high throughput capacity.

The entire apparatus is held in position by means of two specially designed metal supports at the upper and lower ends, which make it possible to assemble or dismantle the apparatus in a few minutes. These supports are also machine-grooved, so as to make all the concentric tubes self-aligning.

Table I illustrates the broad versatility of this apparatus as well as some recommended operating conditions which were found generally useful. For the majority of laboratory fractionations the rate of boiling in the still pot should be maintained at approximately 50% of the initial flooding rate of the recommended packings. This rate of boiling is by no means very critical, since these recommended packings have moderately high fractionation efficiencies at 40 to 80% of their initial flooding rates. Table I also shows that unlimited reflux rates are possible with the micro control reflux regulator. However, for general application, a reflux ratio of approximately 10 to 1 is satisfactory for moderately rapid fractionations. Reflux ratios of 50–1 to 100–1 may also be used when maximum efficiency is required and time is a secondary factor. If the apparatus is used under vacuum the only recommended change is that the packings shown be used as suggested in Table I because of the much higher vapor velocities present during vacuum fractionations. The remainder of the data shown in the table are self-explanatory.

Of the numerous packings and plates reported in the literature, the author prefers 0.16- and 0.3-cm. (0.0625- and 0.125-inch) inside diameter stainless steel helices (*64*) when this metal does not interfere with the material being fractionated, and 0.3- to 0.6-cm. (0.125- to 0.25-inch) glass helices or beryl saddles for packing material where a nonmetallic packing is required. These packing materials have relatively high efficiencies and high throughput over wide rates of boiling. For small-diameter columns of 5 mm. or less, the McMillan (*37*) type of spiral packing has been improved by the author, so that it is the most efficient of the many tested. This improved spiral packing also has an appreciably higher throughput capacity than any of the helix-type packings studied. Owing to its inherent physical characteristics, it cannot be adapted to the larger columns where the helices can be used to advantage.

Efficiency tests on many designs of fractionation columns and packings have been reported in the literature (*1–66*, *68*, *69*). The efficiencies of the majority of packings are reported on the basis of the height of the equivalent theoretical plate (H.E.T.P.) values. While these values are technically correct, they occasionally lead to erroneous inferences. Many

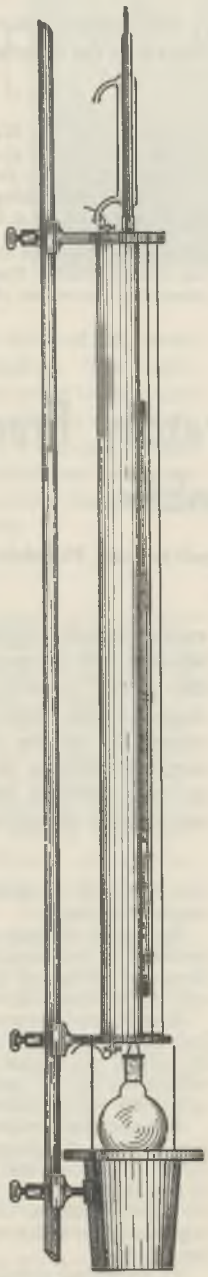


Figure 1

Table I. Physical Operating Characteristics of the Interchangeable Fractionating Columns[a]

Inside diameter of columns, mm.	5	12	25
Length of packings, cm.	90	90	90
Inches	36	36	36
Type of packings	Monel spiral with closed core	1/16-inch stainless - steel helices	1/16-inch stainless - steel helices
H.E.T.P. at total reflux, cm.	1.8	2.2	3.0
Inches	0.7	0.9	1.2
Total number of plates at total reflux	50	41	30
Operating temperature, range, ° C.	30–400	30–400	30–400
° F.	86–752	86–752	86–752
Recommended rate of boiling	50% initial flooding rate	50% initial flooding rate	50% initial flooding rate
Flooding points of above packings (toluene), cc. per min.	3.3	12	46
Recommended fractionation charges, cc.	2–100	100–500	500–5000
Total holdup of column (toluene), cc.	2.	9.2	35
Total holdup of still head and reflux regulator, cc.	0.4	0.5	0.6
Reflux ratios possible with micro-control reflux regulator	Unlimited	Unlimited	Unlimited
Packings for vacuum fractionation	Monel spiral with open core	1/8-inch stainless - steel helices	1/8-inch stainless - steel helices

[a] Data obtained when operating columns at 50% of their initial flooding rates.

H.E.T.P. values reported in the literature were obtained from test columns only 20 to 30 cm. (8 to 12 inches) long. However, Fenske stated (*23*) and showed graphically (*20*, p. 1631) that at total reflux the degree of separation increased logarithmically, not linearly, with the number of perfect plates. Peters (*48*) has shown that the H.E.T.P. varies considerably with different test mixtures when determined under identical test conditions. In addition, the H.E.T.P. values are frequently expressed under total reflux conditions and not under typical working conditions. The author has studied a large number of packings and found that the H.E.T.P. values at total reflux conditions frequently indicated fractionation efficiencies from 25 to 50% higher than they actually were when tested at a reflux ratio of 10 to 1, a practical operating condition. Furthermore, the fractionation efficiency of any packing is usually inversely proportional to the diameter of the column. Consequently, the H.E.T.P. values on one column cannot be readily applied to columns with different dimensions. For these reasons, not only are the H.E.T.P. values given for the different packings described herein when tested by the conventional methods using binary test mixture of *n*-heptane and methyl cyclohexane, but in addition, actual performance fractionations are shown in Figures 6, 7, 8, and 9 which were obtained under typical working conditions. The further significance of these graphs is discussed below.

Numerous types of plates and packing materials have been reported in the literature with the view of improving fractionation efficiency, but very little attention has been given to their practical application under adiabatic conditions. It is a well-established fact that in order to obtain maximum efficiency, any fractionation column must be operated adiabatically (without heat interchange), particularly when fractionating mixtures contain close boiling point components. The large petroleum refinery fractionation columns operate practically adiabatically. This is primarily due to the large throughput capacity of the columns compared to their heat losses. However, the throughput capacity of laboratory fractionation columns is comparatively small and, as a result, external insulation must be used to prevent heat losses. In order to retard column heat losses silvered vacuum-jacketed columns have been used, but even with these the heat losses become significant at elevated temperatures.

Silvered vacuum jackets are almost perfect thermal insulators, but in actual practice an unsilvered strip on the vacuum jackets is

usually used to allow for partial visibility of the packing or plates during fractionation. The heat losses through these unsilvered strips are not very significant at low temperatures (below 100° C.) on large columns. However, these heat losses from small-diameter columns at any temperature, and from large-diameter columns at elevated temperatures, become considerable and greatly reduce the operating range and efficiency of the column. Auxiliary external electrical heaters have also been used in conjunction with the silvered vacuum jackets in an attempt to adjust for these heat losses. These temperature adjustments are usually not sufficiently accurate and are also cumbersome because of the lack of suitable temperature-control equipment.

External heating of laboratory fractionation columns is generally preferred as a means of insulating columns, but there is one serious disadvantage which has not previously been eliminated; previous methods of external heating could not be varied to permit adiabatic fractionation of all types of mixtures. The following two examples illustrate this point:

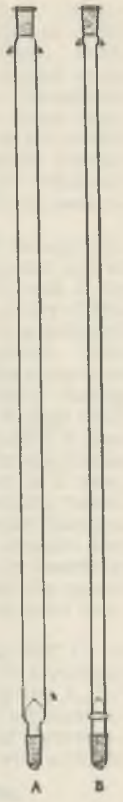


Figure 2

Mixture with Well-Separated Boiling Components. A mixture of 20% of benzene (boiling point 80.2° C.) and 80% toluene (boiling point 110.6° C.) was fractionated. The temperature of the vapor entering the bottom of the fractionation column was 101.8° C. while the temperature of the vapor at the top of the column was 80.3° C. The temperature difference was 21.5° C.

Mixture with Close-Boiling Components. A mixture of 20% of carbon tetrachloride (boiling point 77.0° C.) and 80% of benzene (boiling point 80.2° C.) was fractionated. The temperature of the vapor entering the bottom of the fractionation column was 79.0° C. and at the top of the column was 77.2° C. The temperature difference was 1.8° C.

These two cases illustrate that the temperature gradients of the vapors in the column vary considerably for different mixtures with the same concentration. For this reason the external temperature of the column should be capable not only of being controlled to the temperature of the overhead vapors in the column but also of being varied simultaneously, so that the internal and external gradients of the column are the same.

A rather comprehensive survey of foreign and domestic literature on laboratory fractionation columns revealed no electrical heating device which could be used for the precision control of the external temperature gradient, a condition essential for adiabatic operation. Subsequently the staggered dual heating unit was developed by the author readily to permit essentially adiabatic fractionation of mixtures which have boiling ranges from room temperature up to 400° C. (752° F.). This staggered dual heating unit not only enables one to make accurate temperature adjustments within the apparatus with complete visibility to all parts of the column but also eliminates the necessity of using expensive and fragile silvered vacuum jackets or other types of opaque insulating materials. This heating unit will also permit any type of packing or plate to be operated at increased efficiency by virtue of its inherent design. This design enables the external temperature gradient to be varied along the entire length of the column, so as to be essentially the same as the internal temperature gradient which is determined solely by the mixture being fractionated.

The staggered feature of this dual heating unit will permit nearly any temperature gradient which might be encountered in the fractionation of organic liquids. Typical experimental results (Table II) using a 90-cm. (3-foot) packed column show that the external temperatures can be controlled to that of the internal temperatures of the column regardless of the temperature gradient along the entire length of the column.

Table II. Mixtures Fractionated

Location Inside and Outside of Column Where Temperatures Were Recorded	20% CCl_4 and 80% C_6H_6 (Temperature Gradient 1.8° C.)		20% C_6H_6 and 80% $C_6H_5CH_3$ (Temperature Gradient 21.5° C.)	
	Internal temp. determined by vapor mixture	External temp. controlled by staggered dual heating unit	Internal temp. determined by vapor mixture	External temp. controlled by staggered dual heating unit
	° C.	° C.	° C.	° C.
Top	77.2	77.0	80.3	80.0
Upper quarter	77.6	77.2	83.4	82.8
Middle	78.0	77.4	88.6	88.6
Bottom quarter	78.4	78.2	94.6	93.0
Bottom	79.0	78.6	101.8	100.4

In laboratory fractionations it is impractical to measure the temperatures at all the locations shown in Table II. All that is necessary in practice is to observe the "overhead" vapor temperature and adjust the top external heating unit to this temperature. Then the vapor temperature in the still pot is observed and the lower external heating unit is adjusted approximately to this temperature. All the intervening temperature increments along the entire length of the column will automatically be adjusted to the proper temperature gradient to give substantially adiabatic fractionation. Table II was obtained in this manner. The intervening temperature recordings were specially made to check the accuracy of this staggered dual heating unit but are unnecessary in practice.

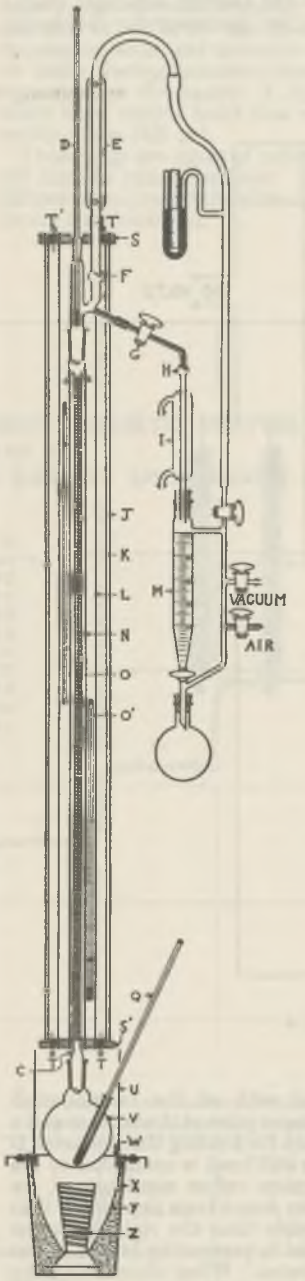


Figure 3

APPARATUS

The essential features of the apparatus are illustrated in Figures 1, 2, 3, and 4.

Figure 1 shows a side view of the apparatus with the stainless-steel jacket in position, leaving the front view unobstructed. Figure 3 shows a cross section of the assembled apparatus with the 5-mm. inside diameter fractionation column in position containing the improved spiral packing. The fractionation columns, *A* and *B* in Figure 2 and *C* in Figure 3, are all interchangeable. Figure 5 shows the entire apparatus mounted in a steel cabinet as it has been used in the author's laboratory. This steel cabinet is not essential to the operation of the fractionation column, since a special stainless-steel jacket is used to protect the apparatus against breakage and excessive drafts in the laboratory during fractionations. This jacket (not shown in photograph) encircles about two thirds of the circumference of the apparatus. Figure 4 shows the staggered dual heating unit which is mounted vertically around the

external circumference of heater jacket, *L*, shown in Figure 3. This heating unit is shown as a separate figure, so as not to confuse its details with the remainder of the apparatus in Figure 3.

CONSTRUCTION DETAILS. Fractionation column *A*, Figure 2, is made of Pyrex brand glass and has upper and lower 19/38 standard-taper joints. It is 25 mm. in inside diameter and has a packed section 90 cm. (36 inches) long. A conical shaped 10-mesh stainless-steel support is used at the bottom of the column to hold the packing in position. External hooks at the top of the column are used to hold the column while inserting it or removing it from the top of the apparatus. Column *B* is similar to *A*, except that it is 12 mm. in inside diameter. The retaining ring at the bottom of the column is used to support it on the recessed hole of support *S'*. Column *C*, Figure 3, is 5 mm. in inside diameter and contains 90 cm. (36 inches) of improved spiral packing. This precision-made spiral packing consists of 1.5-mm. diameter Monel metal rod wound with 6 turns per inch. The center core is made of 1.5-mm. diameter Monel metal rod. Both precision-made spiral packing and glass column are essential for high fractionation efficiency. The center core of the spiral packing is removable for vacuum fractionation. This column is also provided with an additional glass jacket, *N*, to aid in maintaining a uniform temperature gradient in the column during fractionation. A metal retaining ring at the bottom of the column is used to hold jacket *N* in a centralized position and thereby eliminate fragile inner glass seals. This jacket is not necessary for the larger columns, *A* and *B*.

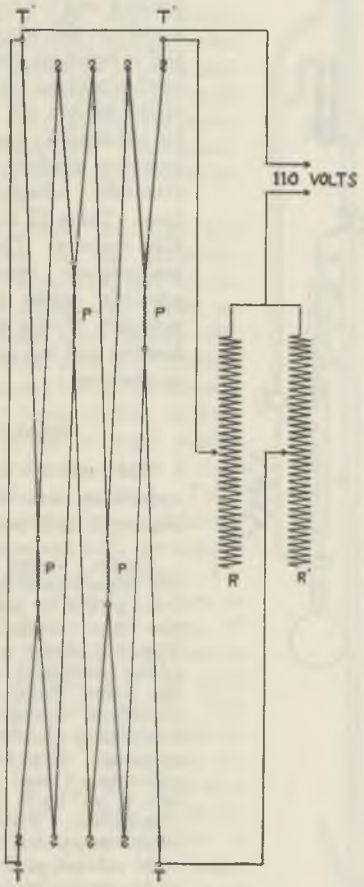


Figure 4

The same still head, *F*, is used with all the fractionation columns. It has a 19/38 standard-taper joint at the bottom and a 10/30 standard-taper joint at the top for holding thermometer *D* at the 75-mm. immersion mark, the still head is connected to the reflux condenser, *E*, and to the micro reflux regulator, *G*, by means of rubber tubing. It has been found from experience that rubber connections are more desirable than the rigid one-piece glass construction; they not only aid in preventing breakage but also facilitate assembling the apparatus. When dismantling the apparatus it is advisable to cut off these rubber connections with a sharp instrument rather than to remove them by hand. The reflux condenser, *E*, has a jacket length of 180 mm. and 12-mm. inside diameter bore.

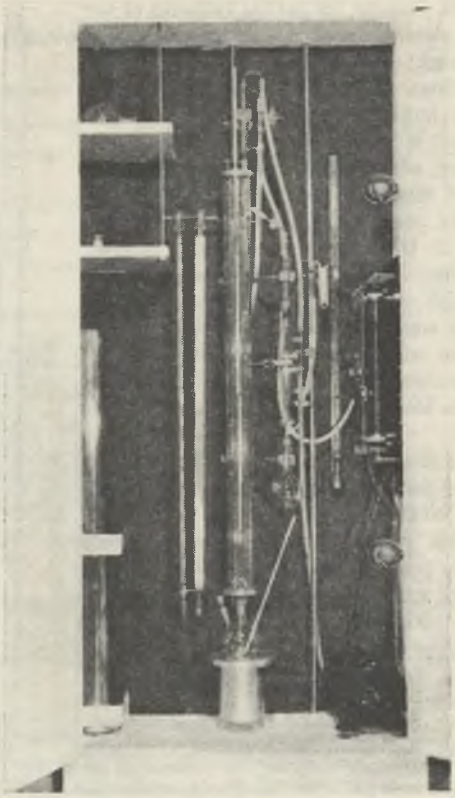


Figure 5. Apparatus Mounted in Steel Cabinet

The micro control reflux regulator, *G*, is a precision-made stopcock which is geometrically grooved around two-thirds the circumference at the plug bore diameter. This stopcock is inclined forward at an angle of 45° and downward at an angle of 30° from the horizontal. The side arms of the reflux regulator are capillary tubing 2 mm. in inside diameter. Drop bulbs, *H* and *F*, are placed on the reflux regulator and still head, respectively, so that the approximate reflux ratios at the top of the column can be readily determined.

The condenser, *I*, used for cooling the hot distillate, has a jacket length of 120 mm. and a bore 6 mm. in inside diameter. The receiver, *M*, which has a capacity of 50 cc. and is graduated in 0.1-cc. divisions, was found the most generally useful size. This receiver is used for both atmospheric pressure and vacuum fractionations. Appropriate stopcocks are used for connections to the vacuum system, air inlet, receiver, and column.

The staggered dual heating unit, illustrated separately in Figure 4, consists of two separate heating units with terminals, *T'T'* and *TT*, respectively. The top and bottom heating units extend the entire length of the apparatus and are overlapped or staggered in the middle third of the column. These heating units are supported vertically and uniformly around the outside circumference of the Pyrex tube support, *L*, by means of S-shaped Monel metal hooks, inserted through small holes near the top and bottom of this support. The upper and lower heating units are insulated from each other by means of small porcelain rings and kept taut with four springs, *P*, which take up the slack when the wires expand on heating. These springs are made of special crucible steel whose elasticity is not affected by moderately high temperatures or the usual corrosive vapors prevalent in the laboratory. Both heating elements are made of No. 22 B. & S. gage Nichrome wire and each has a resistance of 16 ohms and a maximum total heating capacity equivalent to 1500 watts when used on a 110-volt alternating or direct current line.

Slide-wire rheostats, *R* and *R'*, are connected in series with the upper and lower heating units, respectively. By means of these rheostats it is possible to vary the heater jacket temperature from approximately room temperature to as high as 400° C. (752° F.). These rheostats are mounted vertically on a control panel as shown in Figure 5. The third rheostat shown in the

photograph is used for controlling the still-pot heater, *Z*. Rheostats *R* and *R'* are 400 mm. (16 inches) long and 45 mm. (1.75 inches) in diameter and each has a resistance of 105 ohms. Both rheostats are of the heavy-duty type and are water-cooled, so as to maintain constant resistance while in operation. Terminals *T'T'* and *TT* pass through the upper and lower metal supports, *S* and *S'*, respectively, and are insulated from the metal supports by means of flexible herringbone porcelain insulators. Brass connectors are used on the ends of the terminals to facilitate connecting the column heating units to the control rheostats.

The outer Pyrex tube, *K*, aids in the insulation of the apparatus. This tube is 100 cm. long and 7.5 cm. in diameter and is slotted on the upper right side, so as to allow the still head, *F*, to be inserted or removed without dismantling the remainder of the apparatus. In addition to this glass jacket there is a polished stainless-steel jacket, *J*, which also serves as an insulator. This steel jacket surrounds approximately two thirds of the apparatus and leaves the front third open for complete visibility during fractionation. This jacket is made of No. 24 B. & S. gage 18–8 stainless steel with a No. 4 polished finish. This jacket is also slotted on the upper right side and is attached to the apparatus after it is completely assembled. The steel jacket is 3 mm. shorter than all the glass jackets. The outer concentric raised ring on the lower support, *S'*, is machined 3 mm. shorter than the remaining concentric rings on this support. This enables the steel jacket to be readily attached to the apparatus by inserting it from the rear into the outer concentric groove on the upper support, *S*, and then pushing the lower end into the outer concentric groove of the lower support, *S'*.

Thermometers *O* and *O'* are graduated from 0° to 400° C. in 1° divisions for total immersion. They are fastened in a vertical position to the inside wall of the heating unit support, *L*, by metal clamps, so that the temperatures immediately adjacent to the fractionating column are recorded. The still-head thermometer, *D*, is graduated from 0° to 360° C. in 1° divisions with a 10/30 standard-taper joint at the 75-mm. immersion mark. For very accurate fractionations it is advisable to replace this thermometer with one which is graduated in 0.1° or 0.2° C. divisions. Still-pot thermometer *Q* is a standard 75-mm. immersion thermometer graduated from 0° to 400° C. in 1° divisions.

The entire apparatus is supported by means of two specially designed sturdy metal supports, *S* and *S'*, at the top and bottom of the column, respectively. By means of these supports, the fractionation column, heater jacket, insulator jackets, still head, etc., can be rapidly assembled or dismantled. Since the concentric grooves on the supports are carefully machine-finished, all glass parts rest directly upon the supports to make good seals and thus avoid the necessity of using packing materials. The fractionation efficiency of the entire apparatus depends to a considerable extent on the accuracy of these supports because an improper metal-to-glass seal would cause serious convection currents within the apparatus. This effect would become more serious as the temperature rises. These supports greatly tend to eliminate breakage compared to the conventional types of clamps which depend on lateral pressure, and in addition, permit complete visibility in all parts of the fractionation column.

Since this apparatus with its interchangeable columns can conveniently fractionate still-pot charges which vary from 2 to 5000 cc., it has been found useful to have a series of still pots similar to *V*, Figure 3. The following still pots with 19/38 standard-taper joints are recommended: 10, 25, 50, 100, 250, 500, 1000, and 5000 cc. The 10- and 25-cc. still pots are too small to have therometer wells, and therefore are heated indirectly by means of oil baths having immersed thermometers. These still pots are supported on the heater, *X*, by means of removable rings, *W*, which have central holes that vary in size depending on the capacity of the still pot.

These rings are made of polished aluminum 1.5 mm. thick and 125 mm. in outer diameter. Polished aluminum shields, *U*, of different sizes protect the sides and rear of the still pots from drafts during fractionations.

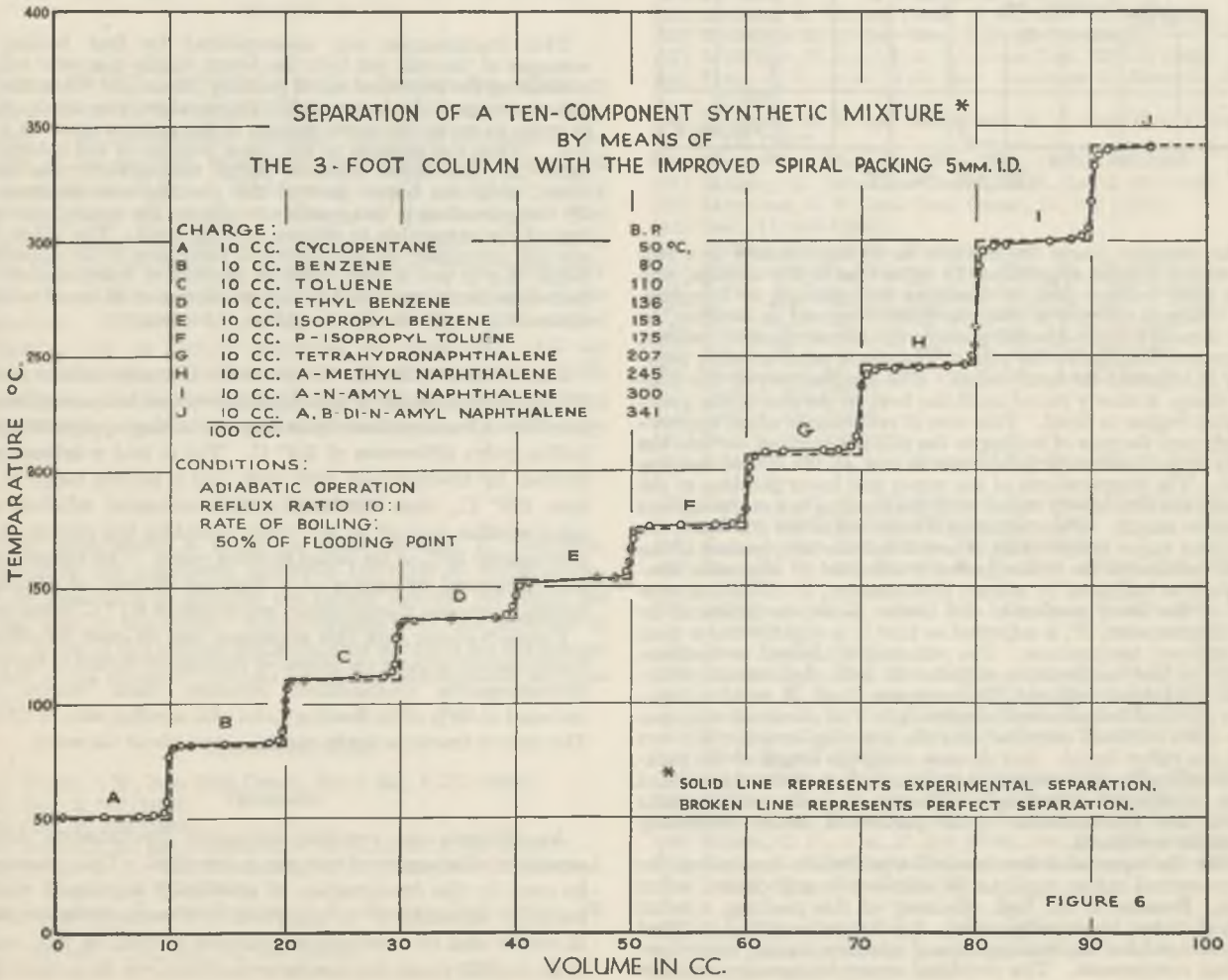
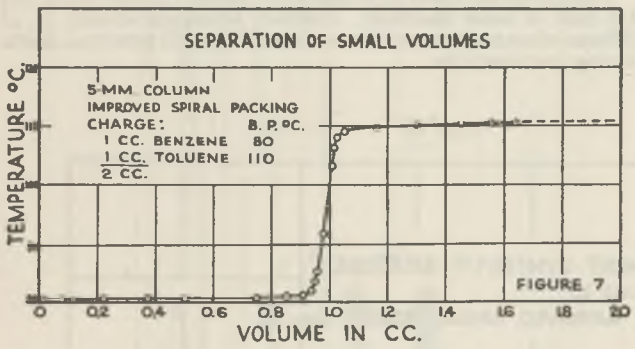


FIGURE 6

Numerous still-pot heaters have been tried, but *X*, designed by the author, was found the most satisfactory because of the very low heat capacity of its construction materials. This enables the still pot to be adjusted with a minimum of temperature lag and to maintain a constant temperature once the still-pot heater is set. The heater consists of an ordinary 500-watt coil heater, *Z*, mounted in a vertical position in a polished aluminum shell, *X*. This shell is 125 mm. in diameter at the top and has a wall thickness of 1.5 mm. A radiant reflector, *Y*, is used to improve heat insulation and is made of No. 30 B. & S. gage polished aluminum. The walls and bottom of the still-pot heater are loosely packed with rock wool to give additional insulation. The temperature is varied by means of a rheostat not shown in the drawings but shown in Figure 5—a heavy-duty water-cooled, slide-wire rheostat with a total resistance of 80 ohms, mounted vertically on the control panel adjacent to the two fractionation column control rheostats.

OPERATION

Figure 6 shows the results of a quantitative fractionation of a complex synthetic organic mixture of liquids when using the 5-mm. inside diameter column, packed with 90 cm. (36 inches) of the improved spiral packing and using a reflux ratio of 10 to 1. The time of fractionation was about 8 hours. The volumes were corrected for holdup. The technique used to obtain this fractionation was developed so that similar fractionations could readily be performed with a minimum of experience or difficulty.



The operator places the mixture to be fractionated in a still pot with a volume approximately twice that of the mixture, and adds some boiling chips to minimize superheating or bumping. The charged still pot is then carefully mounted in position, so that there is a slight upward pressure on the fractionation column to prevent leakage at the joint. The micro control reflux regulator is adjusted for total reflux. The temperature of the still-pot charge is slowly raised until the bottom portion of the packing just begins to flood. This rate of refluxing is noted approximately and the rate of boiling in the still pot reduced, so that the reflux rate is about 50% of what it was at the initial flooding point. The temperatures of the upper and lower portions of the column are then slowly raised until the packing is wet throughout its entire length. When refluxing is observed in the still head, the overhead vapor temperature is noted and the temperature of the upper portion of the heater jacket is adjusted to this same temperature as indicated by column thermometer, *O*. The temperature of the lower portion of the heater jacket, as indicated by the thermometer, *O'*, is adjusted so that it is slightly cooler than the still-pot temperature. The refluxing is allowed to continue after the last temperature adjustment until equilibrium conditions are almost reached. This requires about 10 to 15 minutes after the final temperature adjustment. The overhead temperature then becomes constant and the packing is uniformly wet with the reflux liquid. Any dryness along the length of the packing is indicative of overheating in that portion of the column and must be adjusted accordingly. These temperature adjustments enable the fractionation to be performed under essentially adiabatic conditions.

After the apparatus has reached equilibrium conditions, the micro control reflux regulator is adjusted to any desired reflux ratio. Because of the high efficiency of this packing, a reflux ratio of 10 to 1 is generally useful. A reflux ratio of 50–1 to 100–1 may be used for the fractionation of mixtures having very close-boiling components. The overhead vapor temperature will remain practically constant while the first component is being removed. When this component is practically all removed, the overhead temperature will begin to rise. The upper and lower jacket temperatures are again adjusted, as mentioned above, to raise the external column temperature to adiabatic conditions for the next component. This process is repeated for each component as it is removed. The same procedure is used for atmospheric pressure and vacuum fractionations.

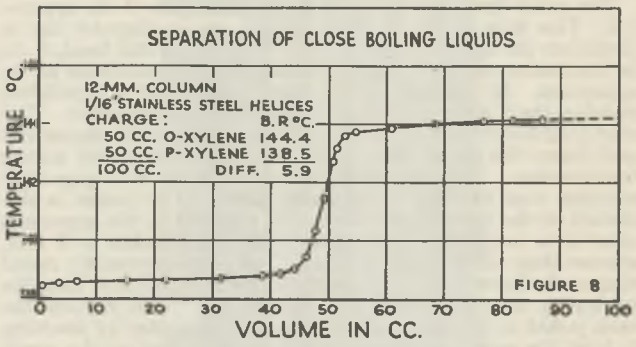


Figure 7 shows that volumes as small as 2 cc. can readily be fractionated. Even though the total holdup of the spiral packing, still head, and micro control reflux regulator is approximately 3 cc. under normal operating conditions, it is possible to fractionate small volumes, owing to the inherent design of the staggered dual heating unit which enables the fractionation column itself to be operated as a "temperature graduated" still pot after it has served its usefulness as a fractionating device.

This fractionation was accomplished by first boiling the contents of the still pot into the 5-mm. inside diameter column containing the improved spiral packing (90 cm.). When the still pot was empty, the lower jacket temperature was slowly raised in order to make the lower portion of the column serve as a still pot. When the packing in the lower portion of the column became dry, the upper external jacket temperature was slowly raised until the upper part of the packing also became dry. By this procedure it was possible to remove the entire liquid content of the apparatus to obtain an 85% yield. The other 15% was lost principally as the vapor still remaining in the apparatus. While this is not a very efficient method of fractionation, the procedure lends itself well to the fractionation of small volumes where only moderate fractionation is necessary.

Figure 8 shows that the 12-mm. inside diameter column packed (90 cm.) with 0.16-cm. ($^1/_{16}$-inch) stainless steel helices can be used for efficient fractionation of mixtures containing components with boiling point differences of 5.9° C. The *o*- and *p*-xylenes were purified by fractionation until each had a boiling range of less than 0.3° C., then combined and fractionated adiabatically, using a reflux ratio of 50 to 1 while operating the column at approximately 50% of its initial flooding point. The temperatures were measured by means of a National Bureau of Standards certified precision thermometer, graduated in 0.1° C. divisions.

Figure 9 shows that this apparatus can be used for efficient fractionations of large variations of volumes by means of the three interchangeable fractionation columns. Each column was operated at 50% of its flooding point with a reflux ratio of 20 to 1. The time of fractionation in each case was about the same.

SUMMARY

An efficient and versatile laboratory fractionation column assembly with improved features is described. This column can be used for the fractionation of practically any liquid mixture normally encountered in analytical and research laboratories. It can be used for fractionating volumes as small as 2 cc. and as large as 5000 cc. having components which boil from room tem-

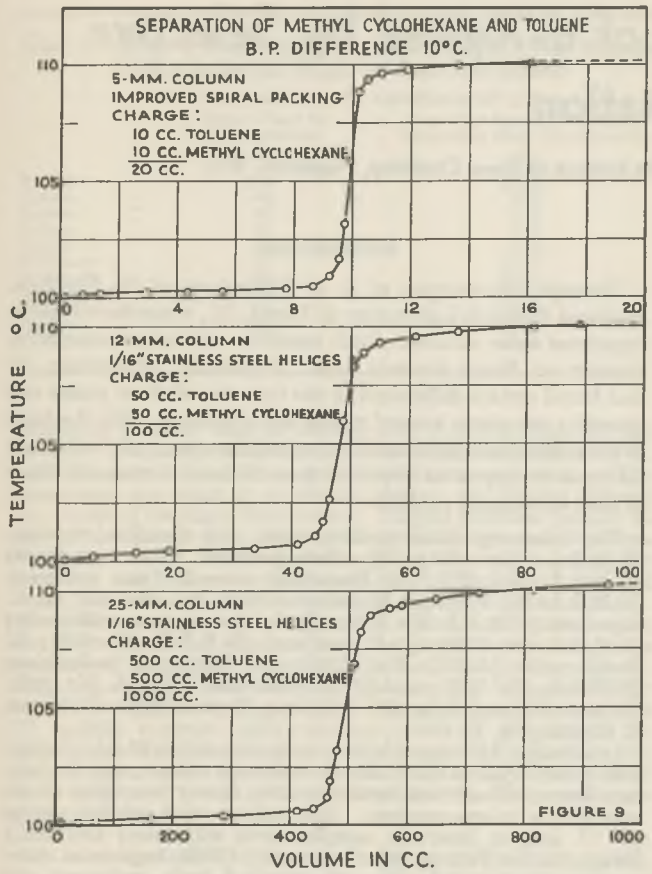


FIGURE 9

perature to 400° C. (752° F.) with a fractionation efficiency equivalent to 30 to 50 (H.E.T.P. 1.8 to 3.0 cm.) theoretical plates. The fractionation efficiency and large throughput capacity of the packings enable complex mixtures to be separated in a minimum of time. Mixtures of *o*- and *p*-xylenes, boiling 5.9° C. apart, can readily be separated quantitatively. The newly developed staggered dual heating unit enables a packed or plate laboratory column to be easily operated under essentially adiabatic conditions with complete visibility to all parts of the apparatus. By means of specially designed supports, the entire apparatus can be rapidly assembled or dismantled. All the above advantages, and the fact that the fractionation column assembly can be readily operated with a minimum of experience and difficulty, make it a generally useful piece of laboratory equipment.

ACKNOWLEDGMENT

This opportunity is taken to thank Stanley P. Reimann, director of the Lankenau Research Institute of Philadelphia, for his generosity in granting the author facilities for doing the initial part of this work. Acknowledgment is also extended to C. C. Ward of the United States Bureau of Mines for his general review of the status of previous laboratory fractionation columns (*67*).

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Quantitative Determination of *d*-Xylose by Selective Fermentation

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A simple microbiological method has been developed which permits the determination of 12 to 50 mg. of xylose in the presence of glucose, mannose, arabinose, and glucuronic acid, with an accuracy of 96 to 104%. It depends on the use of *Hansenula suaveolens* (N.R.R.L. No. 838) which ferments xylose quantitatively but not arabinose, rhamnose, fucose, and glucuronic acid. Inasmuch as N.R.R.L. No. 838 also ferments glucose, the hexoses must be fermented prior to its use. Such fermentation is effected by *Saccharomyces carlsbergensis* (N.R.R.L. No. 379), which has only a very slight action on xylose (an error for which due correction may be made).

DESPITE the biological and industrial importance of *d*-xylose, no specific purely chemical method exists for its quantitative determination. Most straws and hardwoods, in temperate regions, contain over 20% pentosans, which yield largely xylose on hydrolysis. The coniferous woods often contain 7 to 12% xylan, and practically all fibrous raw materials entering the pulp and paper industry (with the exception of cotton and flax) are rich in xylans.

Therefore, waste liquors from chemical pulping operations contain either the precursors of xylose, the sugar itself, or degradation products and derivatives of the sugar. Most commercial pulps also contain xylans.

The presence of xylose in certain liquors obtained on acid hydrolysis prior to pulping (especially those obtained in European rayon plants using straws and hardwoods as raw materials) has led to the use of this sugar as a culture medium in the production of protein feedstuffs and as a source of furfural. Furthermore, in the manufacture of furfural from oat hulls, cottonseed hulls, corncobs, etc., xylans play an important role. It is evident, therefore, that a specific, proximate method for xylose in various industrial liquors and hydrolyzates would be useful.

Since the early analytical work of Councler (*1*) and its modification by Tollens and his co-workers (*4*), a standard group procedure has been used to determine pentosans and pentoses in plant materials. This is the classical furfural method which, however, does not serve to differentiate between xylose and arabinose, or the widely distributed uronic acids. Furthermore, the methylpentoses—e.g., rhamnose—yield methylfurfural, which is not readily separated from its homolog.

The problem of determining xylose and arabinose in admixture was attacked by Hopkins, Peterson, and Fred (*2*) in their studies on the hydrolysis products of root-nodule gums. Working with pure sugar mixtures (containing no uronic acids), they fermented the hexoses quantitatively with appropriate yeasts and then resterilized their solutions, added sterile calcium carbonate, inoculated with pentose-destroying bacteria, and incubated the solutions at 37° C. Under these conditions *Lactobacillus gayonii* (No. 36) destroyed only xylose and *Lactobacillus mannitopoeus* (No. 19) destroyed only arabinose. The final fermentation required 10 days, and satisfactory results could be obtained. These bacteria were also used by Lewis, Brauns, Buchanan, and Kurth in their studies of the sugars obtained from the hydrolysis of redwood bark (*3*). However, this bacterial action was very time-consuming and the organisms required special media and techniques for their maintenance. Thus, a practical method for the quantitative determination of xylose still remained to be devised.

EXPERIMENTAL

Through the courtesy of L. J. Wickerham of the Northern Regional Research Laboratory at Peoria, Ill., a number of microorganisms were obtained which could be maintained readily by transfer on Bacto dextrose agar. Previously, Wickerham (*5*) had found certain differences in the behavior of these yeasts and yeastlike organisms toward xylose and arabinose. On the basis of such differences, qualitative experiments were carried out at the institute to determine which of these (if any) fermented either of the two common pentoses.

The microorganisms studied were: (*a*) *Candida tropicalis* (N.R.R.L. No. 85); (*b*) *Debaryomyces membranae-faciens* (N.R.R.L. No. 927); (*c*) *Hansenula anomala* (var. *sphaerica*) (N.R.R.L. No. 778); (*d*) *H. saturnus* (N.R.R.L. No. 12); (*e*) *H. suaveolens* (N.R.R.L. No. 838); (*f*) *Saccharomyces aceris-sacchari* (N.R.R.L. No. 359); (*g*) *S. muciparis* (N.R.R.L. No. 410); (*h*) *Torula utilis* (A.T.C. No. 8206); (*i*) *Torulopsis molischiana* (N.R.R.L. No. 218); and (*j*) *T. sphaerica* (N.R.R.L. No. 169). (*h*) was obtained from the American Type Culture Collection at Washington, D. C.

In all cases, 100 mg. of xylose (or arabinose) in 25 ml. of water were mixed with 15 ml. of starch-free yeast extract, and the mixture was sterilized and treated with a heavy inoculum of the appropriate microorganism. Incubations were carried out at 30° C. and, at intervals, samples were withdrawn aseptically for qualitative Fehling reduction tests. Of the organisms under experiment, N.R.R.L. No. 85 attacked both arabinose and xylose partially within 92 hours. Organism N.R.R.L. No. 838 fermented xylose completely within that time, and left arabinose seemingly unattacked. The other organisms previously cited showed no appreciable action on either pentose.

As a result of these tests, *Hansenula suaveolens* (N.R.R.L. No. 838) was selected as the most promising xylose fermenter and a series of quantitative experiments was made as in the previous work on fermentation of galactose (*6*), except that N.R.R.L. No. 838 replaced N.R.R.L. No. 379 (*Saccharomyces carlsbergensis*) and that the incubation period was extended to 94 to 96 hours (in place of 48 hours). The experiments showed that xylose (provided not more than 100 mg. in a final volume of 50 ml. were present) was fermented quantitatively. Glucose was also completely fermented. Arabinose was not attacked and *d*-glucurone was attacked very slightly. Mixtures of arabinose and xylose, when treated under these conditions, permitted the recovery of 97 to 99.6% of the arabinose originally present, as indicated by the Munson-Walker cuprous oxide reducing values.

Subsequently it was found that all types of fermentation could be expedited by agitation of cultures on a shaking device during the incubation. Under such conditions xylose was destroyed by N.R.R.L. No. 838 within 24 hours and mannose and glucose were completely fermented within 5 to 6 hours with N.R.R.L. No. 379. Galactose was utilized by N.R.R.L. No. 379 within 24 hours.

In consequence, when no hexoses were present, xylose analyses were carried out as follows: 25 ml. of the sugar solution, containing not over 100 mg. of xylose and not over 225 mg. of total sugars, were added to a 500-ml. Erlenmeyer flask and treated with 15 ml. of 10% starch-free yeast extract. [For the preparation of this cf. (*6*, p. 30). Some recent yeast samples contained added starch. These gave anomalous results.] The mixture was sterilized at 6.8-kg. (15-pound) pressure (121° C.) for 15 minutes, cooled, and inoculated with N.R.R.L. No. 838 (an inoculum so prepared that, in the Cenco-Sheard-Sanford photelometer, it gave a reading of 9–13, when distilled water gave an initial reading of 90 on this instrument). The methods for transferring the organism, and for the preparation and handling of the inoculum were entirely

Table I. Effect of *Hansenula suaveolens* (N.R.R.L. No. 838) on Various Individual Sugars (or Sugar Derivatives)

(24-hour fermentation with agitation at 30° C.)

	Sugar Prior to Fermentation	Sugar (or Sugar Derivative) Recovered after Fermentation
	Mg.	*Mg.*
Xylose	100	None
Xylose	50	0.5
Xylose	50	0.2
Xylose	50	0.3
Xylose	50	1.0
Arabinose	54	54.0
Arabinose	50	50.0
Rhamnose	50	50.8
Rhamnose	25	24.0
Fucose	25	25.0
d-Glucurone	50	46.0

analogous to those used in dealing with N.R.R.L. Nos. 379 and 966 (*6*). The flask was shaken continuously for 24 hours at 30° C. (±0.5°), with a 4-inch stroke and 96 oscillations per minute. The suspension was made up to 100 ml., shaken, and filtered through two layers of hardened filter paper. Aliquot portions (usually 50 ml.) of the filtrate were taken for analysis by the usual Munson-Walker method. Identical, uninoculated controls, in which the inoculum was replaced in each case by 10 ml. of sterile distilled water, were also run under conditions similar to those outlined. The difference between the amount of cuprous oxide obtained in the control and that obtained from the fermented solution was a measure of the xylose content. [The Institute of Paper Chemistry has collected and tabulated data on the Munson-Walker method as applied to sugars other than glucose. These results (*7*) include cuprous oxide–reducing values of (Pfanstiehl) *d*-xylose, *l*-arabinose, fucose, rhamnose, *d*-mannose, *d*-galactose, and sodium glucuronate (purified at the institute). These tables have been used in the present investigation.] The results are given in Table I.

It is evident that xylose is almost completely fermented, whereas arabinose, the methylpentoses (rhamnose and fucose) and *d*-glucurone are practically unattacked by organism N.R.R.L, No. 838. The data establish a basis for the assay of xylose in the presence of other sugars.

When hexose sugars such as mannose and glucose are also present, a preliminary fermentation with organism N.R.R.L. No. 379 must be used. This was carried out as described by Wise and Appling (*6*), except that the mixture was placed in a 500-ml. Erlenmeyer flask (instead of a 125-ml. flask) and shaken continuously for 24 hours (instead of standing for 48 hours) at 30° C. Each experiment was run using two identical portions. At the end of the 24-hour period, one portion was made up to 100 ml., filtered, and the reducing value of an aliquot of the filtrate (aliquot I) was determined by the Munson-Walker method. The other portion was resterilized, inoculated with 10 ml. of N.R.R.L. No. 838, and shaken for another 24 hours at 30° C. A similar aliquot from this second portion (aliquot II) was then analyzed by the Munson-Walker method. The cuprous oxide obtained from I, minus that obtained from II, gave a measure of the xylose present. As found previously (*6*), organism N.R.R.L. No. 379 also has a slight action on xylose.

In more recent experiments, it was shown that about 1 mg. of xylose in a 50-ml. volume was lost during a 24-hour period by the action of N.R.R.L. No. 379, in the absence of hexoses. However, in the presence of a large excess of mannose or glucose, this loss is usually greater, and there are evidently slight fluctuations in the amount of xylose that is destroyed. On the basis of present data, the mean loss of xylose was about 2.0 mg., for which due correction was made in Table II. This table gives the results of xylose determinations in admixture with other compounds. Whenever an asterisk occurs in the final column, no correction has been made because there was no preliminary fermentation with N.R.R.L. No. 379.

To test the method in the hands of workers who had had no contact whatever with its development, but who simply followed the directions given above for the xylose assay, pure sugar mixtures were prepared by Paul Cundy, and the subsequent manipulations were carried out by John McCoy (microbiologist) and Kathleen Murphy (analyst). The authors' thanks are due to these collaborators, whose results are summarized in Table III. These analysts used two different solutions. Solution I contained 1.0003 gram of glucose, 0.5001 gram of xylose, and 0.5000 gram of arabinose. Solution II contained 1.0002 gram of glucose, 0.2500 gram of xylose, and 1.0000 gram of arabinose. In either case the volume was 250 ml., of which $^1/_{10}$ was taken for each experiment. The final aliquots used for the Munson-Walker determination always corresponded to $^1/_{20}$ of the original sugar solutions.

Three experiments *A*, *B*, and *C*, run in duplicate, were made with 25-ml. aliquots, to which 15 ml. of yeast extract were subsequently added and which were then sterilized. To mixture *A* 10 ml. of sterile distilled water were added, the solution was shaken for 24 hours, made up to 100 ml., and filtered, and 50 ml. of the filtrate were taken for the Munson-Walker determination. *A* served as a control for the series because no fermentation had taken place. *B* was treated with 10 ml. of an inoculum of N.R.R.L. No. 379 (which ferments only glucose), the suspension was shaken for 24 hours, made up to 100 ml., and filtered, and 50 ml. of the filtrate were taken for analysis. *C* was treated with 10 ml. of the inoculum of N.R.R.L. No. 379, shaken for 24 hours, resterilized, treated with 10 ml. of an inoculum of N.R.R.L. No. 838, shaken for another 24 hours, made up to 100 ml., and filtered, and 50 ml. of the filtrate were analyzed for reducing sugars.

The weight of cuprous oxide obtained from *A*, minus that obtained from *B* (after fermentation), was a measure of the glucose present. The cuprous oxide obtained from *C*, after fermentation with both N.R.R.L. Nos. 379 and 838, was a measure of the arabinose remaining in the solution. The cuprous oxide obtained from *B*, minus that obtained from *C*, gave a measure of the xylose present.

The data, summarized in Table III, from which cuprous oxide figures have been omitted, are the average results of closely agreeing duplicate determinations. These data indicate not only that the method is serviceable but that in a three-component system, in which the nature of the sugar has been predetermined qualitatively, the method furnishes a means for the quantitative estimation of the other sugars, as well as xylose.

DISCUSSION

Inasmuch as there is no rapid chemical method for the determination of *d*-xylose in sugar mixtures, the selective fermentation of xylose by *Hansenula suaveolens* presents a new tool to the biochemist and the sugar analyst. Hexoses must be fermented first with an appropriate yeast (like *Saccharomyces carlsbergensis*),

Table II. Assay of Xylose in Presence of Other Compounds

Compounds Admixed with Xylose		Xylose Taken	Xylose Fermented Observed	Xylose Fermented Corrected
	Mg.	*Mg.*	*Mg.*	*Mg.*
Two Components				
Glucose	50	25	22.4	24.4
Glucose	50	25	22.5	24.5
Glucose	50	25	23.8	25.8
Glucose	50	10	8.1	10.1
Mannose	50	50	46.2	48.2
Mannose	50	50	46.0	48.0
Mannose	25	50	47.7	49.7
Arabinose	50	50	50.0	*
Arabinose	100	20	19.7	*
Arabinose	10	50	51.0	*
Arabinose	50	50	50.1	*
Sodium sulfate[a]	250	50	50.0	*
Sodium glucuronate	25	50	48.5	*
Sodium glucuronate	17.5	35	34	*
Three Components				
Glucose	25	25	24.5	26.5
Sodium glucuronate	12.5			
Glucose	25	25	24.0	26.0
Arabinose	25			
Four Components				
Glucose	25	25	24.0	26.0
Arabinose	25			
Sodium glucuronate	12.5			

[a] Sodium sulfate was added because this is formed on neutralizing acid hydrolyzates of the polysaccharides with sodium carbonate.

Table III. Assay of Xylose in Presence of Glucose and Arabinose

Sugar	Solution I		Solution II	
	Taken	Found	Taken	Found
	Mg.	*Mg.*	*Mg.*	*Mg.*
Glucose	50.0	50.4	50.0	48.7
Xylose	25.0	23.0	12.5	12.0
Arabinose	25.0	25.1	50.0	49.7

which has minimal action on the pentoses. A continuous shaking technique greatly accelerates all fermentations, and a xylose assay may be completed within 55 hours, usually with an accuracy of 90 to 102% (provided no correction is applied). When due correction is made for xylose losses, the xylose accuracy is usually 96 to 104%. Arabinose, fucose, rhamnose, and glucuronic acid are virtually unaffected by the fermentation.

Sodium sulfate (which may be a component of neutralized sugar hydrolyzates) does not interfere with the analyses.

Inasmuch as xylose is gradually destroyed by hot dilute acids, the assay is not an accurate measure of the xylans originally present in unhydrolyzed natural products, unless allowance is made for such degradation. (Unpublished experiments have shown that when 100 mg. of xylose were heated under reflux with 25 ml. of 2% sulfuric acid, 17% of the sugar was destroyed in 16 hours.) Furthermore, there are no data available on the action of N.R.R.L. No. 838 on the aldobionic acids, which normally occur among the hydrolysis products of the hemicelluloses. For this reason, the method requires further study in its application to the polysaccharides. On the other hand, it should prove useful in determining the actual xylose content of hydrolyzates. The method also serves as a qualitative identification of xylose in mixtures containing glucuronic acid, arabinose, rhamnose, and fucose. The possible application of this method to holocellulose and hemicellulose hydrolyzates is being studied.

ACKNOWLEDGMENT

Grateful acknowledgment is made to L. J. Wickerham of the Northern Regional Research Laboratory for his cooperation in supplying the organisms used in this investigation and to Dorothy Cartier of the institute staff for making a number of the analyses given in Table II.

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Light and Electron Microscopy of Pigments

Resolution and Depth of Field

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MANY of the properties which pigments possess or impart to substances with which they are incorporated depend upon the size, distribution in size, and shape of the pigment particles. Information regarding these characteristics is essential to those who study the properties and uses of pigments. Since the microscope furnishes a direct way of getting such information, it has been, and is, used extensively for this purpose.

Until very recently the light microscope was the only kind available and most of the studies of pigment particles have been made with it. It is not, however, a completely satisfactory instrument for the purpose for two chief reasons: the limit set on the resolving power by diffraction effects and the small depth of field which is a concomitant of large numerical aperture.

RESOLVING POWER

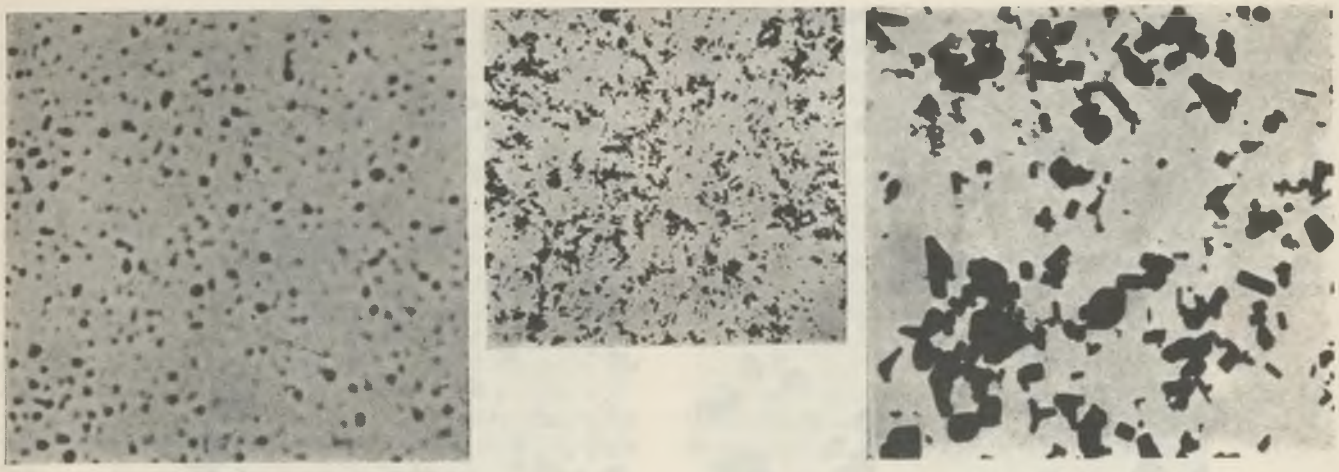
The limit set on the resolving power of light microscopes by diffraction is about 0.11 micron for an ultraviolet microscope using light of 2500 Å. wave length and 0.16 micron for a glass lens microscope using light of 3650 Å. wave length. These values were calculated on the basis of the Rayleigh criterion of resolution and are values which may be approached but not exceeded in actual use of the instruments. Some of the particles of many pigments and nearly all of the particles of others have sizes of this same order. The diffraction effects which limit the resolving power cause the images formed of such particles to be inaccurate, the departure from the truth being greater the smaller the particle. Particles of size below the resolving power of the instrument with which they are viewed all appear the same size regardless of their actual size. Two small particles close together may appear as one. The contours of all images are somewhat diffused and the contours of two particle images may merge where the distance between the two particles is small. Sharp corners appear rounded, the degree of rounding being greater the lower the resolving power of the instrument. This rounding will falsify the shape of particles—for example, images that should be square but appear more and more nearly circular as the particles become smaller.

The effect of a limiting resolving power on the imaging of particles having the silhouettes of regular polygons was investigated both theoretically and experimentally by Borries and Kausche (*1*). Their results may be summarized in the following way. If r is the resolving power of the microscope and D is the diameter of a circle having the same area as the particle silhouette, the ratio of D to r must have the minimum values shown in Table I in order that the image will appear as a polygon rather than as a circle.

As an illustration of the meaning of these results, a particle of square silhouette being viewed with a microscope with a resolving power of 0.2 micron must be about 0.8 micron on a side at the least if the fact that the image should be square and not circular is to be perceived. Since very many pigments have average par-

Table I. Minimum Sizes Relative to Resolving Power for Accurate Images

Particle Silhouette	D/r
Equilateral triangle	2.4
Square	3.9
Hexagon	6.8
Octagon	9.5
Dodecagon	14.8



A B C

Figure 1. Light and Electron Micrographs of Small Particle Size Zinc Oxide

A. Light micrograph, 3855×. *B*. Electron micrograph, 4000×. *C*. Electron micrograph, 16,000×

ticle sizes of the order of 0.5 micron or less, little information regarding the true shape of the particles of such pigments can be obtained with such a microscope. Even the use of an ultraviolet microscope with a resolving power of 0.11 micron improves the situation but little.

The transmission-type electron microscope in its present stage of development has an experimentally demonstrated best resolving power of about 0.002 micron and a resolving power attained with ordinary care in routine practice between 0.004 and 0.007 micron. If, for purposes of illustration, we assume a resolving power of 0.005 micron, we find from Table I that a square particle need be only about 0.02 micron on a side at the least to have its image appear square rather than circular. The great majority of particles of nearly all pigments have diameters greater than this or even greater than the 0.033-micron minimum diameter for a hexagonal shape which we can calculate from Table I. Consequently, the electron microscope yields a more accurate picture of the size and shape of the particles in pigments, particularly of the small-sized ones, than does the light microscope.

COMPARISON MICROGRAPHS

This fact is demonstrated by the micrographs of small particle size zinc oxide in Figure 1 which were made with a light microscope and with an R.C.A. Type B electron microscope.

The first, *A*, was made with a light microscope at 1285× using blue light from a cadmium spark and is reproduced at 3855×. The limiting resolving power of the microscope was 0.16 micron. The particles, whose average diameter is about 0.1 micron, all appear spherical or slightly egg-shaped and have diffuse contours. In contrast, the particle images of the electron micrographs, *B* and *C*, of the same pigment are sharply defined and of a variety of shapes. Figure 1, *B*, at nearly the same magnification (4000×) as *A* contains many images smaller than any in *A*. These appear as mere dots to the eye, but with higher magnification as in *C* at 16,000×, the particles are revealed to be predominantly prismatic in shape.

DEPTH OF FIELD

The effects of the small depth of field which is the inevitable result of the high numerical aperture of a high-power light microscope may be just as serious as low resolving power in obscuring the true shapes and sizes of particles and sometimes in producing a fictitious distribution in size. In making particle-size determinations of the smaller size pigments it is customary, and necessary from a practical standpoint, to make a micrograph of a chosen field of view and measure particle images on an enlarged print or projection of the micrograph. The depth of field of a high-power light microscope used photographically is only 0.1 micron or less. As a result, particles or features of particles which are more than about 0.05 micron from the object plane will not appear in focus in the micrograph. Since the great majority of pigment particles are larger than this, it is a practical impossibility to obtain a light micrograph of a pigment in which the outlines of all particles are in a focus.

Because of the low numerical apertures which are used, the depth of field of electron microscopes as used at present is of the order of several microns. In an electron micrograph properly made, all parts of the outlines of all but the very largest particles in any field of view will be in satisfactory focus.

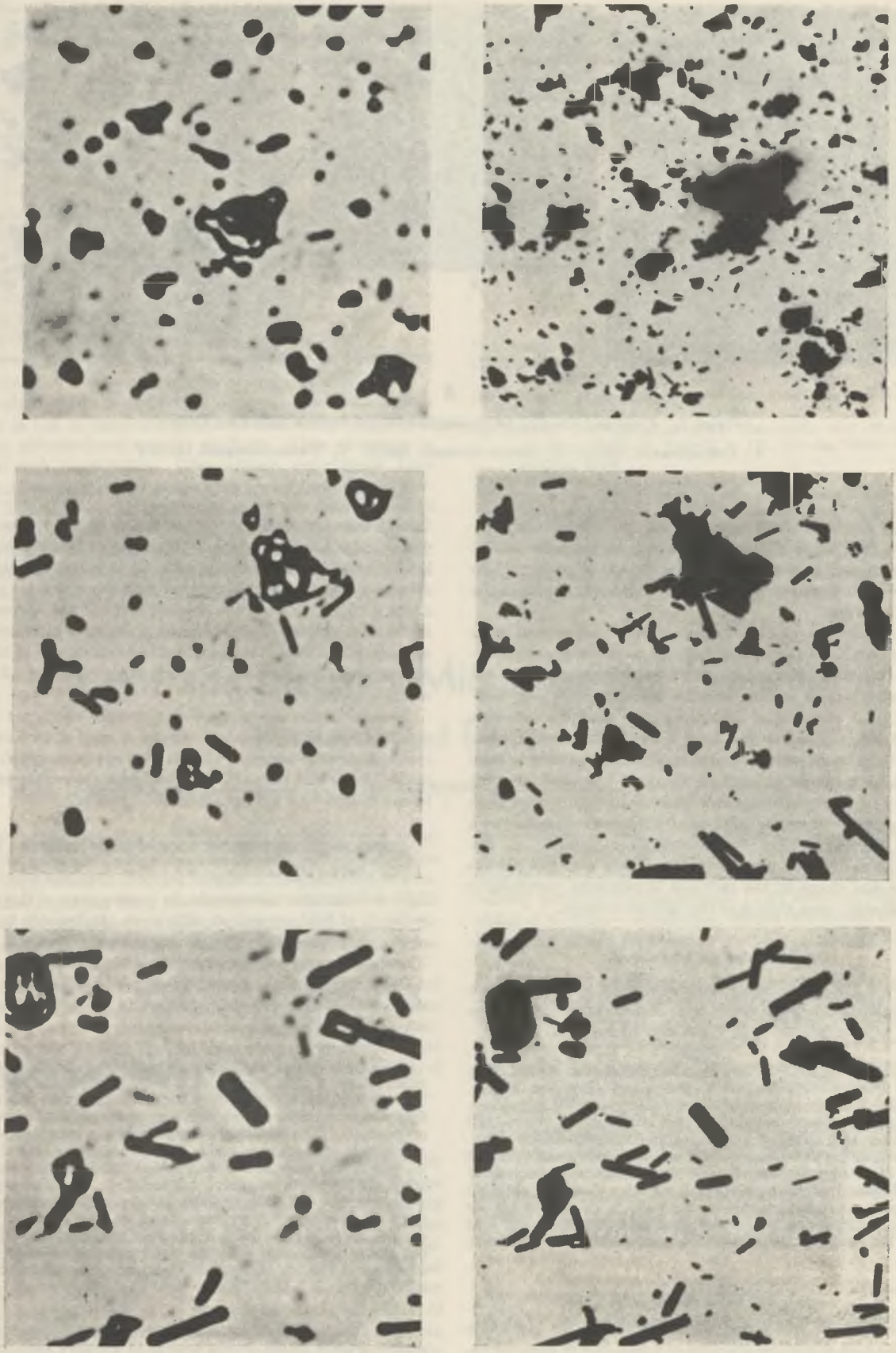
LIGHT AND ELECTRON MICROGRAPHS OF IDENTICAL FIELDS

The differences in images of pigment particles which occur in light and electron micrographs, in consequence of this difference in depth of field, as well as differences attributable to resolving power, may be seen in the micrographs of Figures 2, 3, and 4. These are of progressively larger kinds of zinc oxide.

In each figure there are two micrographs of the same field, one made with the electron microscope and the other with the light microscope. These were made in the following way:

A thin nitrocellulose film containing the dispersed pigment was mounted on a thin brass disk 3 mm. in diameter and having a hole through its center about 0.1 mm. in diameter. An area of the film near the center of this hole was photographed in the electron microscope. The disk was then placed on a microscope slide and completely immersed in cedar oil. A cover slip was then placed on top of the disk and a micrograph made with a light microscope, at the same magnification as before, of the area previously photographed. By the exercise of care in the handling of specimens it was possible to have most of the required areas flat enough to be in good focus in the light micrographs. The resolving power attainable with the light microscope with specimens mounted in this way is probably not so high as with the usual sort of specimens, but the difference is thought to be small.

As in Figure 1, there are present in the electron micrographs small particles which do not appear or are very faint in the light micrographs. This is particularly striking in Figure 3. In each of the three pairs of these comparison micrographs, the large general features of the fields are the same, but the details of the particles are unlike, the differences arising from the difference in resolving power and depth of field of the microscopes. In many



A B

Figure 2 (*above*), Figure 3 (*center*), and Figure 4 (*lower*). Pairs of Light and Electron Micrographs of Identical Fields of Three Different Kinds of Zinc Oxide

A. Light micrographs *B*. Electron micrographs
Magnification, approximately ×5000

cases particles shown clearly in the electron micrograph are so far out of focus in the light micrograph as to be almost invisible. In other cases, the apparent shape of a particle is different in the two micrographs of a pair because part of it is out of focus in the light micrograph. When a particle is in focus in both micrographs of a pair, there usually is a difference in shape in the two images. In one case, a particle (not shown in the figures) which appears to be a sphere 0.4 micron in diameter in the light micrograph had a hexagonal outline of 0.34 by 0.60 micron dimensions n the electron micrograph. In another case an oval image 0.5 by 0.4 micron in the light micrograph was seen to correspond to a needle shape 0.5 by 0.2 micron with a blunt point at either end. In many cases nearly rectangular shapes in the electron micrographs correspond to slightly dumbbell-shaped images in the light micrographs.

The significance of such differences in pigment imaging as are shown here to the micrographical determination of particle size is clear.

Many pigments of small particle size appear in light micrographs to be composed of spherical particles. In many cases electron micrographs reveal that the particle shapes depart considerably from the spherical. These departures will need to be taken into account in any accurate evaluation of particle size by microscopical observation. Further, smaller particles and relatively more small particles appear in electron micrographs than in light micrographs of many pigments, especially in those of small average size. In these cases particle sizes obtained from measurements on electron micrographs will be smaller than previous results. Such particle-size determinations are at present being made on a number of commercial zinc oxides in this laboratory. The average sizes obtained are of the order of 25 to 50% less than previous values obtained with the light microscope.

ACKNOWLEDGMENT

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Titration of Microgram Samples

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A micropipet connected to a leveling bulb is used for a buret which, by remote control, is able to deliver up to 0.05 cu. mm. of standard solution into a drop that is located in the field of view of a microscope for the purpose of continuous observation. The displacement of the meniscus in the buret, too, is determined under the microscope. The micrometer readings may be easily converted to give the customary units of volume and mass, if the burets are calibrated as described and the concentrations of the standard solutions are known. The titrated solution, which may have a volume of 0.05 to 0.5 cu. mm., is contained in an open capillary, one side of which is connected to a plunger device, so that the drop of solution may be made to move back and forth. The eddy currents in the moving drop produce the required stirring. A precision of ±8 to ±15 parts per thousand has been obtained in titrations with acid and with silver nitrate. Observation of a color change in the small volumes of solution is difficult, and so far only colorations obtained in connection with adsorption on microscopic or colloidal particles have been sufficiently sensitive. Other means for the indication of end points are being investigated.

UP TO the present time more dilute solutions have been employed in titrimetric determinations on a microgram scale than are used when working with large quantities (*7*, *9*, *10*). The disadvantages of such procedures are obvious (*5*). Thus, it was decided to develop a technique which permits retaining the customary concentrations of the solutions as it has been done with titrimetric determinations on milligram samples (*5*).

Obviously, greater difficulties have to be overcome on the microgram scale because of the small volumes resulting from adherence to the customary concentrations. Division of the volumes of macroanalysis by one million shows that the capacity of a microgram buret should equal 0.05 cu. mm. and that the volume of the titrated solution at the end point may vary from 0.05 to 0.5 cu. mm. Accurate handling of such volumes requires mechanical manipulators and observation with the aid of a low-power microscope. The general technique (*1*, *2*, *3*), the required microscope with rotating mechanical stage (*2*, *3*, *4*), the simplified manipulator (*2*, *3*), the moist chamber in which the titrations are carried out (*4*), the plunger device (*2*, *3*), the carrier slide (*3*), and the reagent containers (*1*) have been described in preceding papers.

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THE BURET

A micropipet with straight tip as used in qualitative work on the microgram scale (*2*, *3*) serves as a buret. It is mounted in the usual manner in a pipet holder (*h*, Figure 1C) which has been made fast in the clamp of the simplified manipulator (*1*, *3*). The pipet holder is connected by means of rubber tubing of 2- to 3-mm. bore to bulb *a* of the leveling device (Figure 2). Rubber tubing of the same kind is suitable for connecting bulbs *a* and *b* which are approximately half filled with water. Bulb *b* is best made of a separatory funnel of 50-ml. capacity. It is clamped to the rod of a mechanical stand, so that it can be raised and lowered in front of a centimeter scale by means of a rack-and-pinion motion.

The buret proper is shown in Figure 1A. Its nozzle is formed by tip *tp*, shaft *st*, and taper *tr* of the micropipet proper (*4*); the "calibrated tube" is represented by a short length of the shank adjacent to the taper. Actually, only a reference mark, *re*, is provided at a distance of 3 to 5 mm. from the taper, and the displacement of the meniscus is measured with the aid of the eyepiece micrometer of the microscope. The illustration shows only a small portion of the shank which would have to be given a length of 1 meter when drawn to the scale of Figure 1A.

The bore of the shaft of the micropipet is determined by the desired capacity of the buret and by the true diameter of that part of the microscopic field, which is covered by the scale of the eyepiece micrometer. This diameter in turn depends upon the magnification given by the objective. In the experiments reported below, combination of an 8× objective with a screw micrometer eyepiece of Zeiss permitted following the movement of the meniscus over a distance of 2 mm. Thus, a bore of the shank of somewhat less than 0.2 mm. gave the buret a capacity of 0.05 cu. mm.

The dimensions of the nozzle and the tip are by necessity a compromise between precision and efficiency. An opening of the tip 20μ in diameter has been found convenient. It is obvious

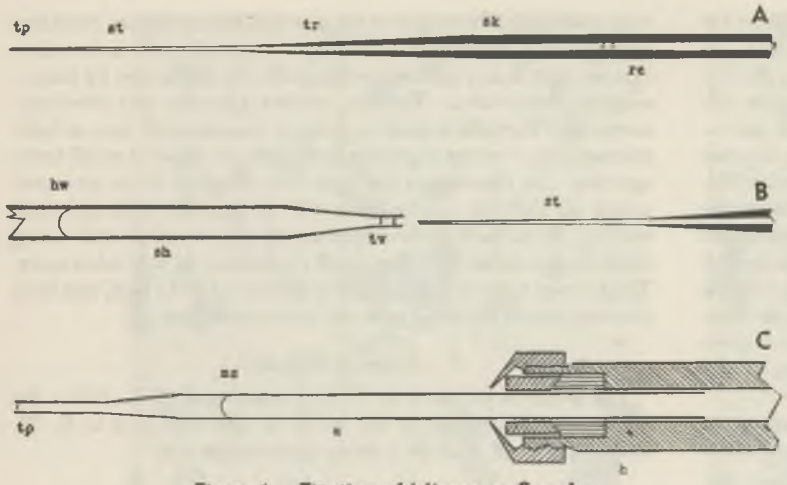

Figure 1. Titration of Microgram Samples

A, buret with remote control, 7 times natural size; *tp*, tip; *st*, shaft; *tr*, taper; *sk*, shank; *re*, reference mark
B, open titration cone, 7 times natural size; *sh*, shank of titration cone; *tv*, titration cone proper; *st*, shaft of buret, *hw*, meniscus of hydraulic water
C, buret, schematic; *tp*, tip; *ms*, meniscus of standard solution; *a*, air column; *h*, pipet holder of metal with rubber washer (shaded horizontally)

that a finer opening would permit a more delicate control of the outflow of standard solution, but would result in greater expenditure of time for each titration.

The operation of the buret relies on the utilization of surface forces, and no stopcock of any kind is required (*2*, *3*). When the tip of the horizontal buret is inserted into an aqueous solution, the liquid would fill the whole tube unless the flow is stopped by raising bulb *b* of the leveling device, so as to give the body of air, which fills the shank of the buret, the pipet holder, the upper half of bulb *a*, and the connecting tubing, a pressure equal to the pressure $2\gamma/R$ produced by the surface tension at the interface, *ms*. As is customary, γ and R signify surface tension and radius of bore at *ms*, respectively. The motion of the advancing meniscus is arrested in this manner when the meniscus arrives at a point approximately 1 to 2 mm. above the reference mark. The "equilibrium pressure" in centimeters of water column, approximately 15 cm., may now be read directly off the leveling device.

When the tip of the buret is withdrawn from the solution, another interface is formed at *tp*. Since the bore, $2r$, at *tp* is considerably smaller than that at *ms*, the surface force originating at *tp* is much stronger than that originating at *ms*. A pressure equal to $2\gamma/r + 2\gamma/R$—i.e., 50 to 150 cm. of water column—is required to expel drops of water from the tip into air. Lower pressures than this affect the curvature of the meniscus at *tp* but cannot produce outflow.

Before starting a titration, leveling bulb *b* is moved into a position giving a pressure higher than equilibrium pressure but lower than required for expelling drops into air. This "operating pressure" can be exactly regulated within a wide range by means of the leveling device, and any desired rate of outflow may be easily obtained. The outflow is then started by inserting the tip of the buret into a solution, and it may be stopped at any time by withdrawing the tip into air.

The burets are prepared from heavy-walled Pyrex tubing having an outer diameter of approximately 6 mm. and a bore of 2 mm. The tubing is placed for 15 minutes in hot chromic-sulfuric acid, then rinsed with tap water, distilled water, and finally alcohol. After drying it is drawn into capillaries of 0.5-mm. outer diameter, which have a bore of approximately 0.2 mm. After the bore is measured, the capillaries are cut into lengths of 20 cm. The nozzles of the burets are best mechanically drawn by means of Rachele's device (*4*). Thus, each length of capillary gives 2 symmetrical burets with fine, open tips. While a stream of clean air is continuously forced through the fine opening, the nozzles of the burets are immersed in smoking hot paraffin and very slowly withdrawn again. Then the very fine ends of the nozzles are snapped off by means of straight forceps, so as to obtain orifices approximately 20μ in diameter. The burets are then laid upon a microscope slide and thus transferred to the stage of a microscope. The portion of the shank adjacent to the taper is inspected for uniformity of bore by measuring the apparent diameter at intervals. Each buret is finally provided with the reference mark on the shank 3 to 5 mm. from the beginning of the taper. Canada balsam is heated on the steam bath until it becomes thick. A trace of the balsam is then used to spin a thread between the two tips of curved forceps. When a thread of the thickness of a hair has been obtained, it is wrapped halfway around the shank of the buret.

The precision obtainable with the burets depends essentially upon a proper choice of objective magnification and micrometer rulings, which makes it possible to utilize nearly the whole length of the scale. If the image of the buret is projected on a ruled screen, correction for the distortion near the edge of the field is easily obtained from comparison with the image of a stage micrometer scale. A displacement of the meniscus equal to nearly twice the length of the scale may be measured, if the zero reading is taken with the meniscus above the reference mark of the buret, whereupon the meniscus is made to move an approximately equal distance below the mark for the final reading.

On an average, approximately 17 scale divisions of each standard solution were used in the titrations of Table I. The maximum reading error was estimated as equal to ± 0.25 division, which corresponds to an average deviation of roughly ± 0.06 division. Considering that four readings were required in each determination of the base-acid ratio, the resulting uncertainty becomes equal to ± 7 parts per thousand when expressed as relative average deviation of a single determination. Somewhat longer columns of standard solutions were used in the titrations listed in Tables II and III, and the reading errors are correspondingly reduced to ± 4 and ± 5 parts per thousand, respectively.

CALIBRATION OF BURET

Since the capacity of the graduated part of the buret will be approximately 0.05 cu. mm., the customary procedure for the calibration does not appear practical. Aside from the necessity of using a highly sensitive microbalance, it is imperative to prevent evaporation of the water delivered by the buret. Thus, it seems preferable to collect the delivered water in a dry capillary and to calculate its volume from the linear measurements of the column formed. With this procedure it is not only permissible but even desirable to perform the calibration with the standard solution which is to be dispensed by the buret.

A 15-cm. length of tubing of 0.2-mm. bore as used in the preparation of burets serves for a calibrating capillary. It is evenly cut 1 cm. from one end, and the diameter of the circular bore is carefully measured on the freshly cut surface of the short piece (*2*). Uniformity of bore is required for a distance of 5 mm. starting at the freshly cut end of the calibrating capillary, and it is tested as described for burets. The other end of the capillary is then tightly sealed into a glass tube, *h* (Figure 3A), of 4-mm. bore and 10-cm. length by means of DeKhotinsky cement.

The calibration is performed in the moist chamber, Figure 3A, which is opened to admit the calibrating capillary and the buret on opposite sides. Standard solutions and water are

Table I. Acid-Base Ratios Using Iodate, Iodide, and Soluble Starch for Indicator

Micrometer Scale Divisions of 0.2 *M*		Ratio,	
NaOH from buret 413	H_2SO_4 from buret 414	Div. NaOH / Div. H_2SO_4	Deviation from Mean
23.4	19.4	1.21	−0.04
20.8	17.0	1.22	−0.03
22.0	17.2	1.28	+0.03
30.2	24.2	1.25	0.00
10.9	8.8	1.24	−0.01
10.9	8.6	1.27	+0.02
6.4	5.2	1.23	−0.02
30.0	23.8	1.26	+0.01
Arithmetical mean		1.25	±0.02 (±15 p.p.th.)

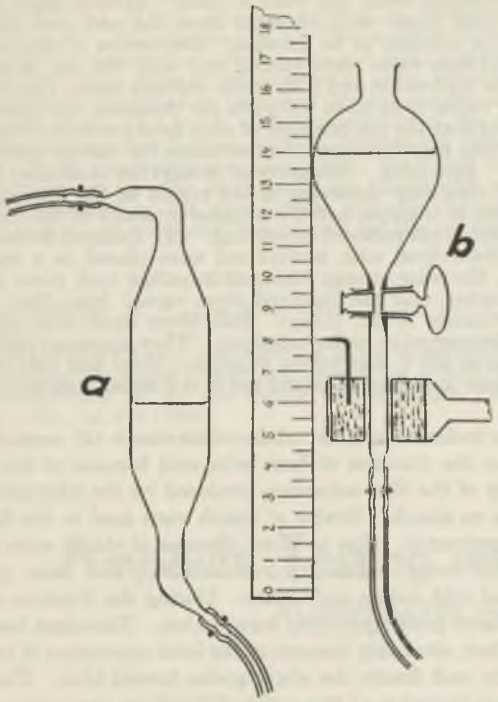

Figure 2. Leveling Bulbs for Use with Micropipets

supplied in reagent containers which are attached by means of Plasticine to the carrier slide. When it may be assumed that tube h, which is held in the clamp of a suitable stand or manipulator, has acquired room temperature, some standard solution is taken into the buret and transferred to the calibrating capillary (*1*, *2*). The operating pressure in the buret is adjusted so as slightly to exceed the equilibrium pressure, and then the tip of the buret is made to touch the inside wall of the calibrating capillary close to its opening. Two menisci form in the calibrating capillary. One remains at the opening, and the other travels toward holder h. When the two menisci are a distance apart, which is approximately equal to the apparent diameter of the bore of the tube (Figure 3B), the buret is withdrawn. The buret is refilled from the reagent container, so as to bring the meniscus close to the reference mark, and the leveling bulb is raised to obtain the selected operating pressure.

Everything is now ready for the calibration of the buret. The reference mark on the shank is brought into the microscopic field, and the distance, b_0, between the meniscus and the mark is measured. The buret is then withdrawn to the right, and the drop of liquid in the calibrating capillary is brought into the center of the field. The buret is now advanced so that its tip appears sharply focused close to the opening of the calibrating capillary. After the distance c_0, Figure 3B, between the two menisci in the calibrating capillary is measured, the tip of the buret is advanced and inserted into the drop of standard solution contained in the calibrating capillary. The meniscus inside the calibrating capillary starts to move. When it arrives at a distance of three fourths of the whole length of the micrometer scale from the fixed meniscus at the opening of the capillary, the buret is withdrawn. This final distance, c_1, Figure 3C, between the menisci in the calibrating capillary is measured, and the final buret reading, b_1, is taken without delay.

Determination of the linear displacement of the meniscus in the calibrating capillary permits calculation of the volume of solution delivered by the buret as the volume of a right circular cylinder of the height $h = c_1 - c_0$ (Figure 3B). No calculations concerning the curved surfaces of the menisci are required. (This is not quite correct, since the curvature of the meniscus at the opening will be slightly changed because of the small increase of pressure of the air enclosed in the capillary and the holder.) The value of one division of the micrometer scale in terms of cubic millimeters of solution delivered by the buret is given by

$$7.854 \times 10^{-10}\, d_c^2 u^3 \frac{c_1 - c_0}{b_1 - b_0}$$

if b_0, b_1, c_0, c_1, and the diameter of the bore of the calibrating, capillary, d_c, are measured in divisions of the micrometer scale and u is the value in microns of one division of this scale.

Calibration of a buret of 0.156-mm. diameter bore with the use of three different calibrating capillaries of 0.120-, 0.124-, and 0.133-mm. bore gave the following results expressed in 10^{-6} cu. mm. of water delivered per division of the micrometer scale: 67, 65, 65, 65, 67, 64, and 64; on the average 65 ± 1 (±16 parts per thousand).

The amount of water left behind on the wall, when the buret is drained, was determined by cutting the buret after calibration and measuring the diameter of the bore near the reference mark. The capacity of the tube was calculated, and comparison with the delivered volume gave the desired information. It was found that the volume of the residual water was approximately 10% of the capacity of a tube of 0.11-mm. diameter bore, so that only 90% of the contained liquid was delivered. The fraction of residual water increased to 12, 13, 20, 24, and 24% when the diameter of the bore was made equal to 0.12, 0.14, 0.16, 0.22, and 0.26 mm., respectively. The rate of flow as indicated by the displacement of the meniscus in the burets varied from 0.005 to 0.08 mm. per second.

A significant drainage error was not observed, in spite of the relatively large amounts of liquid remaining behind on the walls of the capillaries. Obviously, the low rate of drainage employed in calibration and use of these capillary burets makes for a sufficient constancy of the amount of residual liquid. The meniscus travels at a rate of 0.005 to 0.08 mm. per second. The corresponding figures for the horizontal buret of Hybbinette of 1- to

Table II. Acid-Base Titrations Using Same Buret for Both Solutions

Micrometer Scale Divisions of 0.2 *M* NaOH	Micrometer Scale Divisions of 0.2 *M* H_2SO_4	Ratio, Div. NaOH / Div. H_2SO_4	Deviation from Mean
24.6	24.7	1.00	0.00
26.4	26.6	0.99	−0.01
17.1	17.2	0.99	−0.01
20.6	20.4	1.01	+0.01
Arithmetical mean		1.00	±0.008
Macrotitration		1.001	

Table III. Argentometric Titrations Using Same Buret for Both Solutions

Micrometer Scale Divisions of 0.1 *M* NaCl	Micrometer Scale Divisions of 0.1 *M* $AgNO_3$	Ratio, Div. $AgNO_3$ / Div. NaCl	Deviation from Mean
26.6	26.9	1.01	+0.02
32.8	32.4	0.99	0.00
22.2	22.0	0.99	0.00
8.06	7.82	0.97	−0.02
60.6	60.0	0.99	0.00
Arithmetical mean		0.99	±0.008
Macrotitration		0.992	

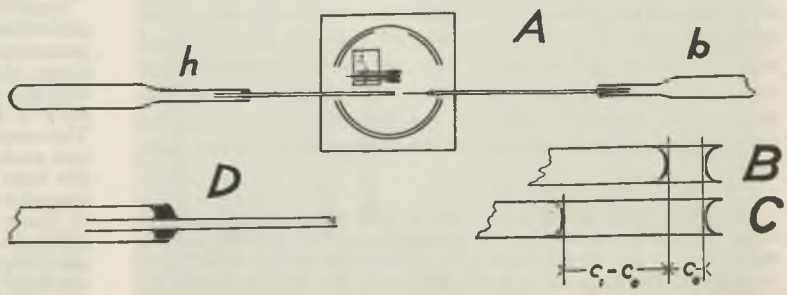

Figure 3. Calibration of Buret

A, B, C, schematic, calibration.... D, use of DeKhotinsky cement for sealing capillary into tube

2-mm. bore in the calibrated portion and the customary macroburet are 0.3 and 5 mm. per second, respectively (*8*, *11*).

STIRRING AND TITRATION VESSEL

It has been pointed out (*5*) that the design of the titration vessel must be the result of a compromise between the conflicting requirements of manipulation and observation of end point. Of the manipulations required, efficient stirring presented the greatest difficulty on the microgram scale and, consequently, became the factor determining the choice of titration vessel.

Considering the volume of the titrated solution, 0.05 to 0.5 cu. mm., stirring by a stream of gas bubbles appeared out of question. A vibrating glass thread (*9*) was tried in various ways while using a capillary cone (*1*, *2*) for a titration vessel, but the results were disappointing. After some experimentation, a method of stirring was perfected which utilizes the turbulence produced in the drop of titrated solution when it is moved back and forth through a capillary tube.

The titration vessel proper is shown in Figure 1B. It is prepared from a Pyrex capillary of approximately 0.5-mm. bore, 0.7-mm. outer diameter, and 20-cm. length. The center portion of the tube is drawn out to a fine capillary of 0.5- to 2-cm. length. The fine capillary is cut at the tapers so that the open titration cones, *tv*, are obtained. Each cone is thus formed on a shank, *sh*, of approximately 10-cm. length. The cones proper are 3 to 5 mm. long, and their bore tapers from 0.5 mm. at the shank to 0.2 mm. at the tip. The far end of shank *sh* is inserted in a pipet holder which is connected to a plunger device or a suitable substitute. It is most convenient to have a second manipulator available, but the pipet holder may be mounted by means of any suitable clamp, so that the open titration cone is introduced into the moist chamber opposite the buret. The meniscus, *hw*, of the hydraulic water is advanced to a point 2 cm. from the titration cone proper before the solution to be titrated is introduced through the opening at the tip of the cone.

The drop in the titration cone is moved back and forth by withdrawing and advancing the plunger acting on the hydraulic water. The displacement of the drop need not be more than 0.5 to 1 mm. A local coloration produced by adding a small volume of strong dye solution is dispelled by 3 to 5 to-and-fro motions of the drop, which then appears homogeneously colored.

INDICATION OF END POINT

So far no practical method has been found for giving the small volume of solution, 0.05 to 0.5 cu. mm., the thickness of layer, 40 mm., required for observation of the end point with the customary concentration of the usual indicators of acidimetry (*5*). Without success were tried in the the titration cone: bromocresol green, bromophenol blue, mixtures of methyl red and methylene blue, and mixtures of methyl orange and indigo carmine, all in concentrations of 10^{-5} to 10^{-4} molar. Capillaries with solutions showing the acid color, the alkaline color, and the middle tint of the indicator were placed alongside the open titration cone for the purpose of comparison. Still, the relative average deviation of a single determination of an acid-base ratio could not be reduced below ±50 parts per thousand, for the color change at the end point appeared merely as a gradual transition of hardly distinguishable shades of gray. An average deviation of ±25 parts per thousand was obtained when 0.5 molar sodium hydroxide was saturated with methyl red and then titrated with 0.25 molar sulfuric acid. This last procedure was used in testing the functioning of the burets, but it cannot be recommended for practical use, since the high concentration of indicator may introduce an error of the order of 50 parts per thousand.

It seemed more promising to use a very small amount of indicator in such a manner that it is concentrated on a small particle suspended in the titrated solution. The color change would then take place on the particle and could easily be observed with the aid of a microscope. After some preliminary experiments it was decided to try silk dyed with methyl red. A thread consisting of a number of fibers was placed between two pieces of cork, and thin sections were cut with a razor. Several short pieces of individual fibers were removed from the cork and transferred into the solution to be titrated. Five series of determinations of acid-base ratio were carried out with the use of 0.5 molar sodium hydroxide and 0.25 molar sulfuric acid. The reproducibility varied from 10 to 100 parts per thousand, and investigation showed that the performance of such dyed particles during actual titrations may fall short of expectation for various reasons:

(1) Bleaching. Silk gives off methyl red in alkaline solutions. Local alkalinity occurring in the region of the fiber causes extraction of the dye, and the original intensity of the red color of the fiber is not restored on stirring. (2) Delayed Action. When silk fibers dyed with methyl red were placed in a solution of pH 8, the color change from red to yellow took place after 1 to 2 minutes, and the required time varied from fiber to fiber. (3) Change of pH Range. Silk fibers dyed with methyl red were immersed in buffer solutions. They appeared red at pH 6, orange at pH 7, and yellow at pH 8. Wool and hair dyed with the same indicator remained red in 0.5 molar sodium hydroxide.

The indicator system iodide-iodate-starch (*6*) seemed promising for the titration of base with acid because of the high intensity of the blue coloration produced by the adsorption of triiodide on starch. Grains of starch were used in the first series of experiments. One to three granules of starch were added to the 0.5 molar sodium hydroxide which had been previously treated with iodide and iodate. During the titration with acid the starch grains gradually turned blue. The edges became colored first, obviously because of the local occurrence of temporary acidity, and finally the whole grains turned blue. The relative average deviation of two series of titrations was as poor as ±90 parts per thousand. Satisfactory reproducibility was obtained, however, with the use of "soluble" starch.

An adsorption indicator was tried in the titration of chloride with silver nitrate. Dichlorofluorescein was used, and the color change was observed on the particles of precipitate. Presence of dextrin improved the sharpness of the end point. The clogging of the burets by the precipitate was overcome by adding a dispersing agent.

Electrometric indication of the end point appears very attractive in view of the difficulties encountered in the observation of color. The problem has been approached in various ways, and a satisfactory solution may be presented in a paper to follow.

TITRATIONS

The manipulations required in titrations are similar to those described in connection with the calibration of burets. The required solutions are held ready inside the moist chamber. The opening of the titration cone and the tip of the buret are made to face one another in the field of vision, and contact of the buret tip with the drop of titrated solution is made and broken by moving the buret, the cone, or the drop in the cone. It is essential that the standard solutions be absolutely clear. The burets, when not in use, are best kept immersed in standard solution, part of which is transferred to this end to small vials with cork stoppers having holes to fit the shanks of the burets. Before use, the burets are rinsed twice with standard solution from the reagent container in the moist cell.

Acid-Base Titration. Five milliliters of approximately 0.5 molar sodium hydroxide was treated with 2 drops of 0.1 molar potassium iodide, 2 drops of 0.1 molar potassium iodate, and 10 drops of a 1% solution of soluble starch. This solution was titrated with approximately 0.25 molar sulfuric acid. The results of a typical series of titrations are compiled in Table I. The amounts of acid and base are given in divisions of the micrometer scale, and, consequently, the listed ratios are functions of the bore of the burets used. Not more than one third of the capacity of the burets was utilized in these titrations. The results of Table II were obtained by using one buret for both standard solutions. Again the volumes were read as divisions of the micrometer scale, but since the bore of the buret remained constant, the base-acid ratio determined in the usual way with 50-ml. burets could be closely reproduced.

Argentometric Titration. To 25 ml. of 0.1 molar sodium chloride solution were added 0.5 ml. of a 2% solution of the

sodium salt of dichlorofluorescein, 2.5 ml. of a 2% solution of dextrin, and 0.2 ml. of 1% aqueous solution of Triton N.E. (Rohm & Haas Co., Philadelphia, Pa.). This was titrated with approximately 0.1 molar silver nitrate. The end point was recognized by observing the color of the particles of silver chloride by means of darkfield illumination furnished by Epi-condenser W of Zeiss. The results of a series of determinations of the ratio volume silver nitrate over volume sodium chloride are listed in Table III. Both solutions were added from the same buret, and the volumes are given in divisions of the micrometer scale. The ratio determined on the macro scale with the customary stopcock burets could be closely reproduced on the microgram scale.

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Ampoule Testing with Manometric Apparatus

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Deterioration of pharmaceutical preparations in sealed ampoules, when accompanied by evolution of carbon dioxide, can be determined by use of the Van Slyke manometric blood gas apparatus. A specially constructed flask is used and the method is similar to that of Van Slyke and Folch for determination of carbon.

PHARMACEUTICALS, packaged in the form of ampoules for parenteral injection, must be subjected to rigid laboratory tests for the purpose of control, both when freshly prepared and after a period of time. When deterioration of a product occurs, on aging, it is often accompanied by the formation of a precipitate or a change in color, either of which is detected by inspection. However, in some cases there is no difference in appearance, since the decomposition is accompanied by no change in color and its products are either in solution or in the gaseous phase. Consequently, inspection shows nothing and the ampoules must be opened and tested. On opening, any gas is lost and in ampoules containing such small quantities as 1 mg. of active ingredient, increased pressure due to decomposition would not be noticed. This paper presents a method for determining the amount of carbon dioxide formed in the above manner. Use is made of the Van Slyke manometric blood gas apparatus, the method being similar to that described by Van Slyke and Folch (*6*) for the determination of carbon. The reader should be thoroughly acquainted with the above-mentioned paper before attempting the work described here. Although determinations of other gases in sealed ampoules have not been attempted, it is believed that these could be accomplished by modifying the methods of Van Slyke (*1–5, 7, 8*) to include use of the flask described below.

APPARATUS

A specially constructed flask replaces the combustion tube used by Van Slyke and Folch, since the carbon dioxide is already present and does not need generation. Figure 1 shows the flask and its relation to the rest of the system. It consists of a 100-ml. round-bottomed flask (*a*, Figure 2) having a ground joint to which is attached a distilling tube, *b*, that is bent so that it may be con-

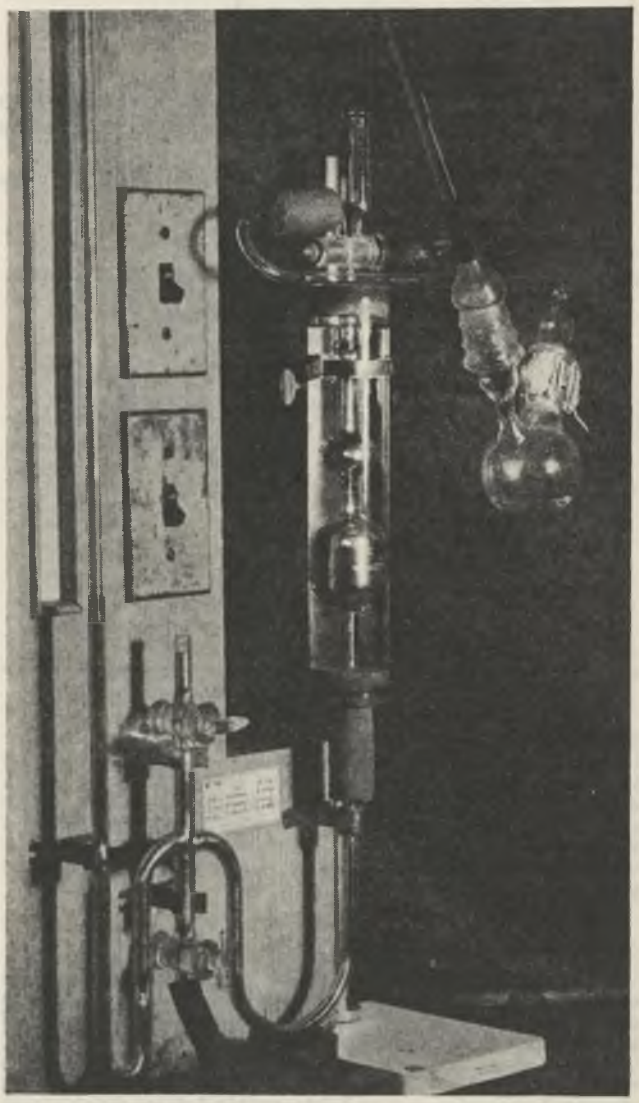

Figure 1. Gas Formation Determination in Ampoules

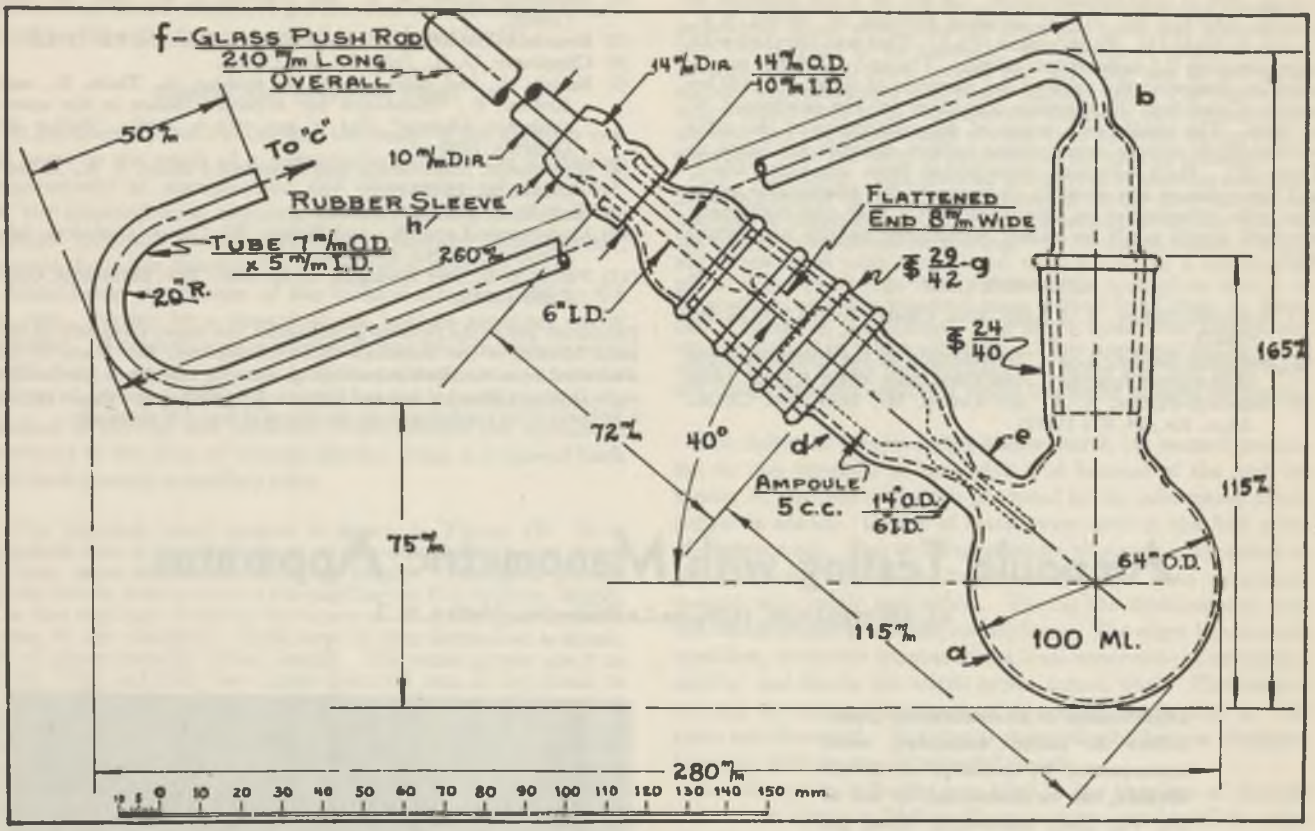

Figure 2. Diagram of Apparatus

Table I. Amounts of Carbon Dioxide Presented with 5-Ml. Ampoules of a Pharmaceutical Product

Temp. °C.	Factors[a]	Blank Values, c, at 2.000 Ml. Mm.	Satisfactory Ampoules P_{CO_2} at 2.000 Ml. Mm.	Satisfactory Ampoules CO_2 Mg.	Unsatisfactory Ampoules P_{CO_2} at 2.000 Ml. Mm.	Unsatisfactory Ampoules CO_2 Mg.
26.5	0.001363	70	0	0.00	..	..
26.5	0.001363	70	..	..	5	0.007
27	0.001360	61	0	0.00	..	..
26.5	0.001363	70	..	..	71.5	0.36
27	0.001360	61	..	..	65	0.32
28	0.001354	61	..	..	63	0.31
27.5	0.001357	61	..	..	64.5	0.32
28	0.001354	61	..	..	51	0.25
28	0.001354	61	..	..	45	0.22
27	0.001360	61	0	0.00	..	..

[a] Obtained from Table I, page 529, *6*.

nected onto the Van Slyke–Folch extraction chamber, *c*. Attached to the side of the flask is a compartment, *d*, for holding the ampoule, which varies with the size of the article to be tested, different flasks being required for each size of ampoule. This compartment is similar in shape to an inverted ampoule having a very thick-walled neck, *e*. To the upper half of the compartment is attached a heavy glass rod plunger, *f*, which passes through the ground joint, *g*, and rubber tubing, *h*, which permits motion.

PROCEDURE

To ensure the proper pH for complete extraction of carbon dioxide, several milliliters of dilute hydrochloric acid are added to the flask, except where addition of acid causes decomposition of the substance in the ampoule with evolution of carbon dioxide. The exact quantity of acid added will depend upon the pH of the material under test. The tip of the ampoule is scratched with a file, care being taken not to produce a track through which gas might escape. The ampoule is then placed in the compartment, neck down, the plunger is attached, and the flask is connected to the extraction chamber as shown in Figure 1. The entire system is evacuated and the air removed from it in the usual manner. The alkaline hydrazine solution (prepared according to the directions of the above authors) is added to the extraction chamber, and the ampoule is broken by depression of the plunger. With the system under reduced pressure, the contents are sucked from the ampoule into the bottom of the flask and mixed with the hydrochloric acid, if used. The carbon dioxide is then absorbed by 25 excursions, after which its pressure is measured in the way described by Van Slyke and Folch. The blank values are obtained by repeating the procedure without having an ampoule present.

Table I gives the data obtained with satisfactory and unsatisfactory ampoules of the same pharmaceutical product, the latter showing evidence of deterioration of the type resulting in evolution of carbon dioxide.

ACKNOWLEDGMENT

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Microdetermination of Water

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The volumetric moisture determination of water by use of the Karl Fischer reagent was adapted to the micro scale. Satisfactory precision was obtained by the use of a small closed titration assembly. The method was tested on a number of materials and data are presented showing that 1 to 25 mg. of water can be determined with a precision of ±20 to 100 micrograms. The method was applied to the determination of moisture in penicillin sodium salt with resultant increase in precision and greater economy of time and labor.

THE determination of water in penicillin salt encounters a number of difficulties and problems. Penicillin sodium salt is heat-labile hygroscopic material containing about 0.5 to 1.5% water when properly prepared. Because of the high concentration of valuable penicillin in the finished salt, it is desirable, if not necessary, to use as small a sample as possible for this determination. A convenient unit is the amount of material contained in one ampoule, which generally lies in the neighborhood of 200 mg. In order to determine the moisture of a sample of this size with a precision of 2 to 5%, a method yielding a precision of 50 to 100 micrograms of water is required. There are a number of liquids and solids containing relatively small amounts of moisture which present similar difficulties in attempting to determine the water content.

The method currently employed by the penicillin industry involves a phosphorus pentoxide–vacuum desiccation of the material over a period ranging from 6 to 9 days, including at least four weighings on the microbalance. Furthermore, the hygroscopicity of the salt causes considerable difficulty in transfer. Two of the greatest objections to this method are the time involved and the large scatter of results from replicate determinations.

It appeared that a method based on the use of the Karl Fischer reagent (*4*) would lend itself to the determination of water in penicillin salt. A review of the literature indicated that the reagent was specific for water (*4, 12*), except for certain metal oxides and carbonyl compounds (*3, 8, 12*), and has been extensively applied to the determination of water in materials miscible with the reagent (*6, 9, 10, 11, 13, 14, 16*). Preliminary tests with penicillin salt (consisting also of the sodium salts of other unknown organic acids) indicated that the material was soluble in the Karl Fischer reagent.

However, the sample sizes used in the reported literature were invariably on a macro scale. Within the last few years, a number of improvements in the method, involving the back-titration technique (*1*) and the application of the dead stop method of Foulk and Bowden (*5*) using a polarized pair of platinum electrodes in conjunction with electrometric apparatus (*7, 15*), resulted in a precision which was potentially of the order of magnitude desired (several micrograms).

A simple reduction in sample size, while maintaining existing techniques, resulted in very poor precision due to the fact that the presence or entrance of moisture, which is negligible on macro scale, was generally of the same order of magnitude as the moisture in the sample. A refinement in the method and technique was necessary in order to adapt the method to a micro scale.

APPARATUS

Figure 1 shows a detailed photograph of the titration assembly, designed for the purpose of reducing the entrance of moisture to such an extent that its effect is negligible.

The titration vessel consists of a 16-mm. diameter Pyrex test tube cut to about 50-mm. length in order to reduce the surface and air space. Two 26-gage platinum wires, serving as electrodes, are fused through the bottom of the vessel. A tightly fitting serum-bottle sleeved rubber stopper is fitted into the top of the vessel and rolled over the outside. The end of a Schilling-type microburet is fitted with a 22-gage hypodermic syringe needle, the end of which is forced through the rubber stopper into the titration vessel. The hypodermic needle shown at an angle is a 27-gage needle which acts as a valve during titration and equalizes the pressure in the titration vessel. The platinum electrodes are connected with an electrometric apparatus by means of the clamps shown.

The electrometric apparatus used is that described by McKinney and Hall (*7*), except for a slight modification. The resistor, *R*-2, indicated in their diagram, was changed to a 4000-ohm variable resistance. A similar unit, now available commercially, has been tested with this method and found satisfactory. (Serfass control unit, Catalog No. 4937-F, Arthur H. Thomas Co., Philadelphia, Pa.)

The air supply to the burets must be thoroughly dried before entering the system and the latter closed entirely from the atmosphere when not in use. Thereby, the concentration of the solutions can be maintained within 1% for at least 10 days.

REAGENTS

KARL FISCHER REAGENT. This reagent should be prepared in large batches and used only after storage of several weeks. Its composition is the same as that used by Smith, Bryant, and Mitchell (*13*).

BACK-TITRATING SOLUTION. This solution is prepared by adding 2 to 5 grams of distilled water to 1 liter of anhydrous methanol. Synthetic methanol, anhydrous, available commercially, is satisfactory for this purpose.

STANDARD WATER SOLUTION. An ethanol solution of approximately 92 to 95% ethanol is prepared and the water content is accurately determined by density.

STANDARDIZATION AND CALCULATION

The back-titrating solution is standardized against the Karl Fischer reagent and the volume ratio determined. The water equivalent of the Karl Fischer reagent is determined by titrating against the standard water solution as outlined under "Procedure" below.

The per cent moisture of a sample is calculated from the data as follows:

$$\text{Per cent moisture} = \frac{(v_1 - v_2 \times f) \times e \times 100}{w}$$

where v_1 = volume of Karl Fischer reagent used
v_2 = volume of back-titrating solution used
f = volume ratio of Karl Fischer reagent to back-titrating solution found in standardization
w = weight of sample
e = water equivalent of Karl Fischer reagent

$$= \frac{w_s \times c}{v_1' - v_2' f}$$

where w_s = weight of standard water solution
c = per cent water by weight in standard water solution
v_1' = volume of Karl Fischer reagent used
v_2 = volume of back-titrating solution used

PROCEDURE

The titration vessels and rubber stoppers are dried in a forced draft oven at 105° C. and stored over phosphorus pentoxide until ready for use. Solid samples are weighed on a balance, transferred rapidly to the titration vessel, and stoppered immediately. Liquid samples are introduced into the sealed vessel by means of a hypodermic syringe and the weight is determined. An equalizing valve needle is inserted into the rubber diaphragm. The Karl Fischer reagent is then added by means of the hypodermic needle attached to the buret until discoloration of the reagent

Table I. Determination of Moisture in Ethanol Standard

(By density: 7.65 ± 0.05%)

Sample Weight	Water Found	
Mg.	*Mg.*	%
312.7	23.98	7.67
238.5	18.27	7.66
296.3	22.67	7.65
305.7	23.40	7.65
306.1	23.40	7.64
298.8	22.83	7.64
326.3	24.74	7.58
267.3	20.49	7.67
299.2	22.79	7.62
279.3	21.33	7.64
	Av. ($\bar{x}$)	7.64$_3$
	Standard deviation (σ)	0.027

ceases, and an excess (5 to 10%) is added. The needles are withdrawn and the vessel is shaken until solids are dissolved. Both the needle attached to the methanol buret and the equalizing needle are inserted into the vessel and the electrodes are attached to the electrometric apparatus. The aqueous methanol solution is added with gentle agitation until solution begins to decolorize. Owing to the elastic junction between the hypodermic needle and the rubber stopper, an effective agitation is accomplished by gentle tapping of the titration vessel. Addition is continued dropwise until the electric eye indicator closes.

The container for the finished penicillin salt consists of a 25-ml. bottle capped with a rubber stopper and therefore can be used directly without transfer of material. Figure 2 shows a detailed photograph of the assembly. The Karl Fischer reagent is added as described above, the needles are withdrawn, and the vessel is shaken until solids are dissolved. Two 23-gage hypodermic syringe needles are inserted through the stopper and two lengths (approximately 7.5 cm., 3 inches, each) of 30-gage platinum wire are passed through the needles. The needles are then withdrawn, leaving the platinum wires inserted in the penicillin bottle. The equalizing needle valve is inserted into the stopper, the needle of the buret containing the back-titrating solution inserted into the bottle, and the electrode connected to the electrometric apparatus by means of the clamps.

DISCUSSION OF RESULTS

The results are expressed as per cent by weight of water found in the sample and the dispersions of the values are also expressed as per cent of the mean value. In order to avoid confusion in the following discussion, %, unmodified, indicates per cent by weight of moisture, and % (of the mean) indicates the precision of the mean expressed as per cent.

The precision of any group of data was calculated as a plus or minus range expressed as per cent (of the mean). This range was determined by a procedure described in the A.S.T.M. manual on presentation of data (*2*). In this case, dealing with several observations on a single sample, the limit $\pm a\sigma$ was applied for $P_s = 0.99$.

The assumptions underlying the application of the A.S.T.M. procedure can be considered valid for the groups of data consisting of 10 samples each. However, in the case of those groups which consist of 4 samples each (Table IV), the assumptions must be applied with caution. In these cases, the precision calculations were used for comparison purposes only.

A redistilled sample of ethanol, diluted with water to about 92% ethanol, was analyzed by density determination using a Bureau of Standards hydrometer and found to contain 7.65 ± 0.05% water. Ten replicate determinations of water by the microtitration method are tabulated in Table I. The precision of the mean of the water content in the ethanol standard is in the neighborhood of ±0.4% (of the mean).

Three liquids were chosen at random in the laboratory and the water content was determined by means of the microtitration method. The water content of these liquids is usually determined with great difficulty. The results are presented in Table II. The precision of the means in these three cases are 0.7% (of the mean), 1.5% (of the mean), and 1.2% (of the mean), respectively.

In order to check the method for solids, three materials were chosen at random from the laboratory and the data presented in Table III. In these cases, the precisions were 0.2% (of the mean), 0.4% (of the mean), and 0.4% (of the mean), respectively.

Three lots of penicillin sodium salt were selected from production and four bottles from each lot analyzed by the microtitration method. Simultaneously, four additional bottles from one of the lots (No. 66) were analyzed for water by the standard phosphorus pentoxide desiccation method. The results are presented in Table IV.

The precisions by the titration method were 5.3% (of the mean), 3.2% (of the mean), and 2.8% (of the mean) with an average of about 3.8%. The precision by the vacuum drying method was in the neighborhood of 14.8% (of the mean) or about one fourth of that by the titration method.

The mean value of the moisture content by the vacuum drying method is significantly greater than the mean by the Karl Fischer titration method. In general, the vacuum drying method will give a higher value of the moisture content, since the transfer of the salt from the container to the weighing bottle will invariably result in absorption of moisture from the air by the penicillin salt which is highly hygroscopic. This source of error is entirely eliminated in the procedure recommended here, since no transfer is involved. For the same reason, the dispersion of the results obtained by the vacuum drying method will be greater

Figure 1. Titrating Vessel

Figure 2. Penicillin Bottle Used as Titrating Vessel

Table II. Determination of Moisture in Liquids

Compound	Sample Weight	Water Found	
	Mg.	*Mg.*	%
2,3-Butylene diacetate	272.1	5.92	2.18
	276.4	6.02	2.18
	290.5	6.35	2.19
	264.2	5.73	2.17
	260.6	5.63	2.16
	307.2	6.68	2.17
	232.3	4.97	2.14
	288.5	6.22	2.16
	273.9	5.96	2.17
	302.8	6.55	2.16
		Av. ($\bar{x}$)	2.16$_8$
	Standard deviation (σ)		0.014
Tetramethyldioxane	278.5	2.17	0.78
	231.6	1.84	0.80
	213.6	1.67	0.78
	238.2	1.84	0.77
	241.3	1.88	0.78
	242.0	1.94	0.80
	260.0	2.04	0.78
	240.5	1.88	0.78
	230.3	1.84	0.80
	258.2	2.04	0.79
		Av. ($\bar{x}$)	0.78$_8$
	Standard deviation (σ)		0.011
Acetic acid (glacial)	239.0	1.61	0.67
	283.0	1.88	0.66
	273.4	1.84	0.67
	283.6	1.88	0.66
	257.4	1.68	0.65
	279.5	1.88	0.67
	259.5	1.74	0.67
	249.0	1.68	0.67
	265.6	1.74	0.66
	277.6	1.84	0.66
		Av. ($\bar{x}$)	0.66$_4$
	Standard deviation (σ)		0.007

than that obtained by the titration method, since the amount of moisture picked up from the air is variable and dependent upon the technique.

CONCLUSIONS

The necessity of determining moisture content of penicillin salt led to the development of a chemical micromethod for moisture determination utilizing the Karl Fischer reagent. A refinement of the method was achieved which led to a degree of accuracy suitable for most purposes.

It was demonstrated that a precision corresponding to a level of 20 to 100 micrograms can consistently be obtained and thus a sample containing 2 to 10 mg. of moisture can be analyzed with a precision similar to usual analytical chemical methods. Most liquids or solids soluble in the reagent can be analyzed by this technique and the method was proved satisfactory for a variety of compounds and preparations including aliphatic, aromatic, and heterocyclic organic compounds, enzyme preparations, etc.

With respect to the moisture determination of the industrial product penicillin sodium salt, a great increase in precision and saving in labor and time were obtained.

It is possible that the technique described herein can be adapted to other volumetric determinations on a micro scale.

Table III. Determination of Moisture in Solids

Compound	Sample Weight	Water Found	
	Mg.	*Mg.*	%
Phloroglucinol	154.7	33.50	21.65
	197.5	42.88	21.71
	230.1	49.92	21.69
	78.9	17.12	21.70
	99.1	21.47	21.66
	109.7	23.88	21.77
	153.8	33.17	21.64
	131.6	28.61	21.74
	108.5	23.58	21.73
	98.7	21.47	21.75
		Av. ($\bar{x}$)	21.70$_4$
	Standard deviation (σ)		0.044
Trypsin	66.1	5.36	8.11
	76.0	6.11	8.05
	45.7	3.70	8.10
	62.4	5.04	8.07
	51.3	4.15	8.09
	59.3	4.81	8.11
	58.9	4.74	8.05
	25.1	2.03	8.09
	60.0	4.84	8.07
	59.5	4.84	8.13
		Av. ($\bar{x}$)	8.08$_7$
	Standard deviation (σ)		0.027
Citric acid	114.2	5.82	5.10
	63.7	3.24	5.09
	111.5	5.72	5.13
	106.1	5.43	5.12
	84.2	4.32	5.13
	147.1	7.52	5.11
	105.6	5.36	5.08
	111.0	5.66	5.10
	106.4	5.43	5.10
	110.2	5.66	5.13
		Av. ($\bar{x}$)	5.10$_3$
	Standard deviation (σ)		0.018

ACKNOWLEDGMENT

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Table IV. Determination of Moisture in Penicillin Sodium Salt

Lot No.	Sample Weight	Water Found	
	Mg.	*Mg.*	%
	A. Titration with Karl Fischer Reagent		
J43	466	6.08	1.30
	466	6.21	1.33
	466	6.21	1.33
	466	6.31	1.35
		Av. ($\bar{x}$)	1.32$_8$
	Standard deviation (σ)		0.021
66	227	3.57	1.57
	227	3.53	1.56
	227	3.57	1.57
	227	3.60	1.59
		Av. ($\bar{x}$)	1.57$_3$
	Standard deviation (σ)		0.013
126	484	2.52	0.52
	484	2.56	0.53
	484	2.52	0.52
	484	2.52	0.52
		Av. ($\bar{x}$)	0.52$_3$
	Standard deviation (σ)		0.005
	B. Vacuum Drying over P_2O_5		
66	227	4.02	1.77
	227	3.64	1.60
	227	3.91	1.72
	227	3.95	1.74
		Av. ($\bar{x}$)	1.70$_8$
	Standard deviation (σ)		0.07$_8$

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NOTES ON ANALYTICAL PROCEDURES

Metallo-Organic Complexes in Organic Analysis

Some Qualitative Tests for Amines

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SALICYLALDEHYDE in ammonia or primary amine solution precipitates nickel, the precipitant being the imine (*2*). The reaction of 5-nitrosalicylaldehyde is similar to that of salicylaldehyde, except that ammonia solutions produce a precipitate only after a number of hours, the exact time depending upon the ammonia concentration. However, a solution of 5-nitrosalicylaldehyde and nickel ion, when added to a primary amine, produces a immediate precipitation. This is the basis for the qualitative test for primary amines.

Secondary amines, when allowed to react with carbon disulfide, produce dialkyldithiocarbamates (*1*). When the reaction is allowed to proceed in the presence of nickel ion, a precipitate of the carbamate with nickel is formed. Primary amines fail to produce a similar precipitation under the conditions of the test.

PRIMARY AMINES. *Reagent.* To 15 ml. of triethanolamine are added 0.5 gram of 5-nitrosalicylaldehyde (m.p. 124–5° C.), and about 25 ml. of water, and the aldehyde is brought into solution. Then 0.5 gram of nickel chloride hexahydrate dissolved in a few milliliters of water is added, and the total volume is brought to 100 ml. The reagent, now ready for use, is stable for long periods of time. If the triethanolamine contains some ethanolamine, it may be necessary to add another 0.5 gram of the aldehyde and filter off the resulting precipitate.

Procedure. To 5 ml. of water are added 1 or 2 drops of the amine to be tested. If necessary a drop or two of concentrated hydrochloric acid may be added to dissolve the amine. When 0.5 to 1 ml. of the amine solution is added to 2 to 3 ml. of the reagent in a test tube, an almost immediate precipitation indicates the presence of a primary amine. The presence of a slight turbidity indicates primary amine as an impurity only. Aromatic amines generally require 2 to 3 minutes for the test.

Sensitivity. The sensitivity of the test is almost constant on a molar basis from one amine to another. When the concentration of the amine in the test solution is 0.02 *M*, a test is obtained. Below 0.02 *M*, the test loses its distinctiveness.

SECONDARY AMINES. *Reagent.* To 0.5 gram of nickel chloride hexahydrate in 100 ml. of water is added enough carbon disulfide so that, after shaking, a globule of the carbon disulfide is left on the bottom of the bottle. The reagent is stable for long periods of time if tightly stoppered. If all the carbon disulfide evaporates, more must be added.

Procedure. An aqueous solution of the amine or its hydrochloride is prepared as under the test for primary amines. To 1 ml. of the reagent in a test tube is added 0.5 to 1 ml. of concentrated ammonium hydroxide, followed by 0.5 to 1 ml. of the amine solution. A precipitate indicates a secondary amine. A slight turbidity is an indication of secondary amine as an impurity.

Sensitivity. As in the test for primary amines, this test has about the same sensitivity on a molar basis for all amines. The lower limit is about 0.01 *M* in the test solution.

Each test was applied to the following amines:

Primary	Secondary	Tertiary
Methyl	Dimethyl	Trimethyl
Ethyl	Diethyl	Triethyl
n-Propyl	Di-*n*-propyl	Tributyl
Isopropyl	Di-*n*-butyl	Dimethylaniline
n-Butyl	*n*-Methylaniline	Triethanolamine
Isobutyl	Diethanolamine	Pyridine
s-Butyl	Piperidine	
Cyclohexyl	Morpholine	
Aniline		
Ethanolamine		
Toluidine		
Naphthylamine		

The tests operated satisfactorily, without exception. In some cases amines of one class gave slight tests for amines of another, indicating the presence of impurities.

INTERFERENCES. Hydroxylamine, hydrazine, and its derivatives where only one nitrogen is substituted, give a positive test for primary amine. Urea and other amides do not give primary amine tests. No common interference was found for the test for secondary amines.

High concentrations of ammonia or tertiary amines invalidate the test for primary amines by rendering the precipitate soluble. High concentrations of secondary amine produce an anomalous precipitate. In general, the tolerances are 75 moles of ammonia, 20 moles of secondary, and 25 moles of tertiary amine to one of primary amine. The test for secondary amines operates in all concentrations of ammonia, and primary and tertiary amines.

Many water-insoluble organic compounds readily dissolve the precipitates, necessitating the removal of such solvents if possible.

SUMMARY

Tests for primary and secondary amines which succeed in the presence of relatively high concentrations of amines of other classes and ammonia are described. The former are based upon the reaction of the amine with 5-nitrosalicylaldehyde, accompanied by precipitation of the nickel complex with the imine and the latter upon the reaction of the secondary amine with carbon disulfide accompanied by precipitation of the nickel complex with the carbamate.

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A Simple Polarographic Cell

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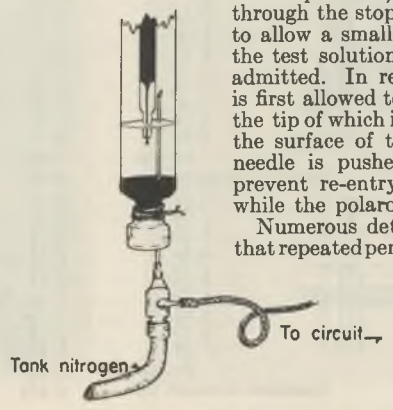
POLAROGRAPHIC cells for small volumes of solution usually call for delicate glass-blowing jobs or platinum wire-to-glass seals which are often unsatisfactory. A satisfactory cell which avoids these difficulties is shown in the diagram.

The cell consists of a 5-ml. syringe barrel (of the rubber plunger type) with the rubber stopper held securely in place by a few strands of small copper wire. A 20-gage, 5-cm. (2-inch) stainless steel needle with a piece of wire soldered to its base serves to admit nitrogen and complete the circuit. The unit is mounted on a small clamp (not shown in the diagram).



In operation, the needle is inserted through the stopper but slightly off center to allow a small amount of mercury and the test solution to be more conveniently admitted. In removing oxygen, nitrogen is first allowed to flow through the needle, the tip of which is then withdrawn beneath the surface of the liquid. After this, the needle is pushed above the surface to prevent re-entry of oxygen from the air while the polarogram is taken.

Numerous determinations have shown that repeated penetrations of the needle into the stopper do not cause it to leak. The cell has proved to be very convenient for small volumes (1 to 2 ml.) and practically unbreakable.

Determination of Copper in Aluminum Alloys

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A DIRECT acid solution procedure for the determination of copper in aluminum alloys by electrodeposition gives excellent results. The method employs a mixture of perchloric acid and nitric acid to effect solution of the alloy. This procedure is particularly suitable for continuous analyses in which the presence of salts introduced by caustic solution methods followed by acidification (*1*, *2*) is objectionable, and for certain types of alloys, such as high-silicon alloys, for complete solution of the sample proceeds readily.

Chromium, manganese, and nickel can be determined after electrolysis. The determination of chromium can be very readily accomplished on a separate sample by oxidation with perchloric acid. Manganese can also be estimated, after solution of the alloy, by the usual oxidation procedures. Nickel is best determined on the electrolyte by gravimetric or colorimetric methods using dimethylglyoxime reagent.

Table I. Results of Acid Solution Method

Alloy Designations	N.B.S. Values	Alkali Procedure	Acid Procedure
N.B.S. 85	4.11	..	4.10 4.11
N.B.S. 86a	7.65	..	7.66 7.65 7.66
N.B.S. 86b	7.87	..	7.88 7.88 7.87 7.87
Alcoa 52-S	..	0.30 0.30	0.31 0.31
Alcoa 2-S	..	0.16 0.16	0.16 0.16
Alcoa 195	..	4.10	4.08
Alcoa X-195 (1)	..	4.47	4.45
Alcoa X-195 (2)	..	4.36	4.36
Alcoa 355	..	1.30	1.33
Alcoa B-195	..	4.30	4.31

Results obtained are shown in Table I, compared to the alkali method. Deposits are bright and adherent. The procedure is applicable to the complete range of copper occurring in aluminum alloys. Elements which deposit in strong acid solutions interfere—for example, bismuth in screw machine products. Fortunately, they are seldom encountered. If present, a subsequent purification of the deposit is necessary.

PROCEDURE. Dissolve 1 gram of aluminum alloy drillings in a 200-ml. electrolytic beaker by adding 20 ml. of perchloric acid (70%), 5 ml. of distilled water, and 5 ml. of concentrated nitric acid (specific gravity 1.42). Warm gently after the initial vigorous reaction decreases to complete the solution. A second 5-ml. addition of water often facilitates completion of the reaction. Add 4 ml. of concentrated nitric acid, and 2 ml. of dilute sulfuric acid (1 to 1) and bring to a boil to expel nitrogen oxides. Wash down the sides of the beaker with distilled water at room temperature and dilute to 150 ml. Electrolyze for 30 to 40 minutes, using a current of air to agitate the electrolyte. A platinum wire spiral serves as the anode and a platinum gauze cylinder 4.4 cm. (1.75 inches) in diameter and 5 cm. (2 inches) in length as the cathode. The current is adjusted to 2 to 3 amperes at the beginning of the electrodeposition and need not be adjusted afterward. The completeness of the deposit can be tested by use of a sodium sulfide solution.

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OPINIONS expressed are those of the author and should not necessarily be construed to represent the views of the Navy Department.

Packing Support for Laboratory Fractionating Columns

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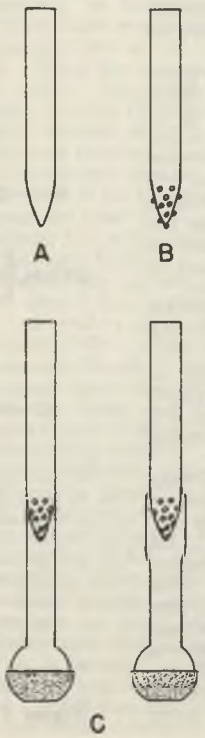
ONE of the more difficult problems in building a packed laboratory fractionating column is the construction of a satisfactory support for the packing. Various designs have been proposed, but all have one or more disadvantages: flooding because of insufficient capacity, corrosion of construction materials, or ease of breaking.

These disadvantages have been eliminated in a packing support which the writer has been using for several years. This support has the additional advantage that the rate of return of the liquid to the still pot is easily observed, since the liquid flows from a single drip point. Packing supports have been constructed for columns ranging in size from 7 to 52 mm. in diameter and packed with packing ranging from 0.24-cm. ($^3/_{32}$-inch) glass helices to 0.6-cm. (0.25-inch) Berl saddles. The glass blowing involved in the construction of the packing support is of only moderate difficulty and consists of two steps.

A short piece of tubing the same diameter as the column is shrunk and pulled down in a flame to give a tube having a conical shaped closed end, the cone having the same wall thickness as the tube, *A*. The tip of the cone is then heated quickly with a fine pointed flame, such as that obtained with a No. 1 tip of a Hoke-Jewel torch and a small bulb is partially blown out. This procedure is repeated in such a manner that the surface of the cone is covered with uniformly spaced small bulbs, *B*.



The partially blown out bulbs are sanded off with coarse sand paper, giving a perforated cone. These perforations should be small enough, so that the packing does not fall through, and have an area equal to the cross-sectional area of the column. Some practice is required to produce small holes for small packing. The cone is carefully heated until all the holes have been fire-polished and then annealed. The prepared section, *B*, is attached to the bottom part of the column with a ring seal, *C*. The completed unit, *C*, is then sealed to the tube which is the main part of the column.

The best method of making this ring seal is as follows: The parts are clamped horizontally in such a position that they touch where the seal is to be made. The portion of glass in the vicinity of the seal is kept hot during the next operation by a relatively cool gas-air flame of a blast lamp. If the flame is too hot the glass will sag. A portion of the outer tube is heated with a fine pointed flame and pushed against the inner tube with a file or iron wire. This is repeated along the entire length of the seal. The seal is worked out in the usual manner and annealed in a flame, or preferably in a furnace.

Water Vapor and the McLeod Type of Vacuum Gage

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THE McLeod gage after 70 years (*3*) is still the ultimate standard for vacuum measurement and is used in calibration of all other vacuum gages. Noncondensable gases, such as hydrogen, cause no error in readings as they do with electrical gages but condensable vapors, such as water or alcohol, cause serious error. Types of errors encountered and methods for avoiding them are discussed in terms of the portable swivel type (*1*, *2*).

Turning the swivel gage cuts off a definite volume, V, of the rarefied air at the unknown pressure of the vacuum system, P, and compresses it to smaller volume, v, at higher pressure p. V is the combined volume of the measuring bulb and the center tube (closed-end or measuring capillary). The value of p is equal to the difference in level of the mercury in the center tube and the right outside tube (compensating capillary). Boyle's law is used in calibration of the scale.

During compression the pressure-to-volume relation of Boyle applies only to noncondensable gases. With condensable vapors there is much greater contraction, since they withstand only a small degree of compression before condensing. If the pressure, p, exerted on the vapor trapped in the center tube exceeds the condensation pressure (varies with temperature), the vapor condenses, and as a result of the greater contraction in volume in the center tube, the mercury will indicate a far better vacuum than actually exists. No simple correction or multiplication factor can take account of this.

SPECIFIC EFFECTS OF WATER VAPOR

CONDENSATION, HIGHER PRESSURE RANGE. Compare two gages of about the same scale length, one having a range of 0 to 5000 microns and the other 0 to 700 microns connected to the same vacuum system. With water present, different readings will be obtained but neither will be correct, because it is the difference in level of the mercury, p, in the two capillaries which sets up the pressure resulting in condensation of the moisture. At 24° C. the vapor pressure of water is 22 mm.; in other words, if p is 22 mm. or more, the vapor will be condensed. This point on the measuring capillary, corresponding to difference in level equal to the vapor pressure, we may call the condensation point.

In the wide-range gage, the condensation point corresponds on the scale to a pressure of about 100 microns; on the narrow-range gage, to 11 microns. Consequently, if there is largely water vapor in the gage, at say a pressure of 250 microns, and very little air, say 2 microns, the vapor will condense to liquid (invisibly small in amount) and the contraction in volume will cause the mercury to rise in the measuring capillary to within 22 mm. from the top, corresponding to the vapor pressure of the condensed water.

Thus the wide-range instrument will indicate 100 microns and the narrow-range, 11 microns. Actual total pressure (air and water vapor combined), 252 microns, is far above the pressure indicated by either gage. All the operator knows is that the air alone is not over 11 microns; if it were more, it would keep the mercury from rising so high in the center tube.

When there is enough air in the system to yield readings above 100 microns, the two gages come into closer agreement but both readings are still in error. For example, take a case of 24° C., 100 microns of air and 1500 microns of water vapor, yielding a total pressure of 1600 microns. With the wider range scale, p would comprise 22 mm. for the compressed air plus 22 mm. for the partial pressure of water vapor under compression which sets up saturated conditions. Thus 44 mm. of scale length would indicate a vacuum of 450 microns. Similarly, with the narrower range scale (0 to 700), p would comprise 51 mm. for the air and 22 mm. more for water vapor, the total of 73 mm. indicating 200 microns. Both gages would give exactly these same fictitious readings in microns with actual pressures even as high as 5000 microns, were only 100 microns due to air and 4900 to water vapor. Similarly, with 100 microns of air and 150 microns of vapor, a total of 250, the wide-range gage would read about 175 microns and the narrow-range 200 microns. Drying the gages will correct these errors.

OUTGASSING, LOW PRESSURE RANGE. At total pressures below the condensation point, condensation cannot occur and accurate readings are obtainable. If water vapor coming slowly from the glass or mercury in the gage, however, is not pumped away completely enough to establish equilibrium with the system, a fictitiously high reading is obtained. Generally, this is at pressures below 10 microns; this error also may be corrected by drying. Outgassing of noncondensables from glass or rubber can be corrected only by pumping sufficiently long. At very high vacuum rubber connections must be eliminated.

CORRECTION OF ERRORS

ERRORS OF CONDENSATION. *Hot Gage Method.* This is applicable where the range of operating pressures is such that at some reasonably high temperature the condensation point will exceed all pressures to be encountered. For example, at 38° C. the 5000-micron range gage will give true total pressures up to 500 microns; at 48° C., up to 2000 microns; and the 700-micron range gage up to 250 microns. At 66° C., either gage will give true total pressures up to the maximum of its range. This method will not always function at highest vacuum—e.g., where moisture from the mercury itself is not pumped away rapidly enough, errors of fictitiously high readings due to outgassing are magnified.

Air Flush Method. This is applicable where a high-capacity vacuum pump is used.

A T-tube is placed in the line to the gage. By means of a stopcock or a pinchcock, the gage is closed off from the vacuum sys-

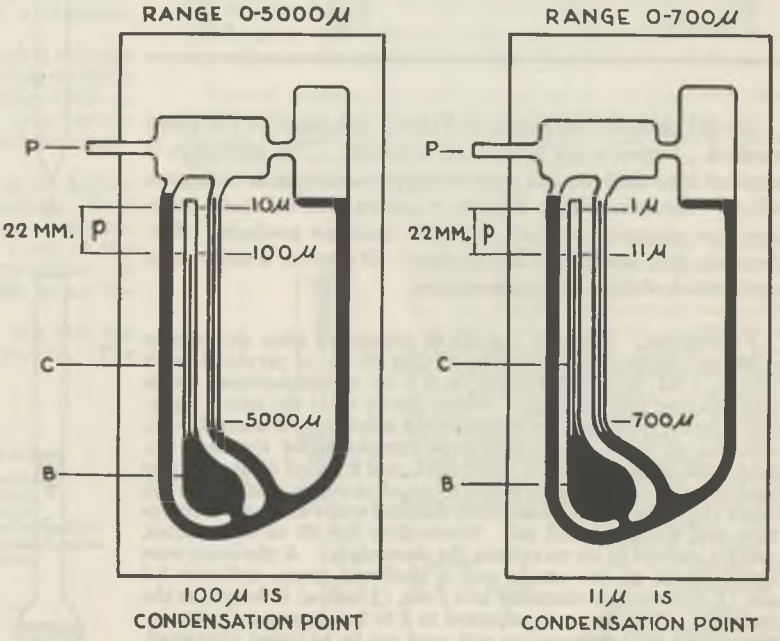

Figure 1. Comparative Condensation Errors in Gages of Different Scale Range

tem and air is admitted to the gage through the T-tube. The gage is then evacuated again to flush out most of the water vapor. In about 2 minutes, a reading will be found higher than the original before flushing in proportion to the amount of water vapor which was present initially. Further readings gradually become lower again as vapor diffuses back into the gage. Each time a reading close to the true total pressure in the system is desired, the gage must be flushed. This method is not applicable at higher vacuum.

ERRORS OF BOTH CONDENSATION AND OUTGASSING. *Chemical Trap.* This is the simplest method of all.

A glass tube large enough in diameter to cause no undue restriction of flow is filled with a chemical selected on the basis of a vapor pressure well below the minimum to be encountered in the high-vacuum system. By suitable selection, chemicals for vapors, such as alcohol and oil, which cause similar errors may be removed. An indicator chemical may be used, so that time for replacement may be determined readily. At highest vacuum, say at pressures below 0.1 micron, it may be necessary to cool the chemical trap.

Freezing Trap. A low-temperature condenser kept cold by means of dry ice, liquid air, or liquid nitrogen may be used for freezing out the moisture at a properly low vapor pressure. The necessity of continually supplying the refrigerant and frequently removing the condensate makes this method less convenient.

CERTAINTY OF ACCURATE READINGS

The chemical or freezing trap causes true total pressures to be indicated, even though only dry air is in the gage at all times. This air accumulates in quantity, so that its pressure exactly balances the total pressure of water vapor and air in the system on the other side of the trap. Even traces of moisture will cause some degree of error and new gages (or old ones that have just been cleaned) may require several days under vacuum, protected by a moisture trap, for complete removal of moisture in order to ensure accurate readings. The gage must be kept protected and only dry air admitted to it.

Since the wider the range of the gage the higher the condensation point, a moist gage of wide range can be used up to higher pressures than one of the narrow range before errors of condensation arise. A condensable vapor trap is recommended to exclude all vapors permanently, the chemical type being the most practicable. Using two gages of different scale range and obtaining identical readings is the best means of knowing with certainty that readings are accurate. With a McLeod gage of the recording type for producing a continuous record of vacuum, a proper trap for moisture is indispensable.

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Microdetection of Sulfur in Insoluble Sulfates and in Organic Compounds

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THE procedures described are based upon the evolution of nitrogen in the sulfide-catalyzed reaction between sodium azide and iodine (*1*, *3*, *4*):

$$3I_2 + 6NaN_3 \xrightarrow{K_2S \text{ or } Ag_2S} 6NaI + 3N_2$$

The reaction is catalyzed by metallic thiocyanates, thiosulfates, and sulfides, whether soluble or insoluble in water, and also by organic compounds which contain the group —C(=S)— or —C(—SH)—, and some others with active sulfur—e.g., sulfathiazole—to which the test may be applied without preliminary treatment (*2*, *5*). To apply this test to the detection of sulfur in insoluble sulfates or nonvolatile organic compounds, a minute quantity of substance, in a molten globule of potassium hydroxide on the tip of a piece of fine copper wire, is heated in the reducing zone of a flame, whereby sulfur in any combination is converted to potassium sulfide. Organic compounds whose volatility excludes this procedure are pyrolyzed in a capillary tube internally coated with silver; the resulting silver sulfide serves to catalyze the iodine-azide reaction. It is advisable to test first directly—i.e., without a preliminary decomposition—and then, if the result is negative, to use one of the procedures described below, as in this way some indication as to the manner of combination of sulfur in an organic compound may be obtained (*2*, *5*). Application of all three procedures requires only a small amount of substance and can be completed in 5 minutes.

PROCEDURES

REAGENT. Dissolve 1.0 gram of sodium azide in 50 ml. of 0.5 *N* iodine solution.

1. DETECTION OF SULFUR IN NONVOLATILE SUBSTANCES. Clean the end of a piece of thin copper wire (0.1 to 0.2 mm.) with nitric acid or emery paper to remove sulfide, and wash with distilled water. Dip the clean end of the wire into a saturated solution of potassium hydroxide in water, and pass it through the flame of an alcohol lamp, so as to evaporate the water and leave a small bead of molten potassium hydroxide at or near the end. [A gas flame may be used if sufficiently low in sulfur to yield a negative blank test. It is an advantage of the test, especially in mineralogical field work, that an alcohol lamp (whose flame is certain to be sulfur-free) will serve.] If the bead does not form at the tip, the wire beyond the bead may be cut off. Bring the bead of alkali into contact with the substance to be tested. Introduce the bead and adhering sample into the flame, moving the bead slowly into the reducing cone, and after a few seconds allow it to cool near the wick. Use one of the following manipulations to detect the sulfide formed.

Cut off that portion of the wire covered by the bead of alkali and place it on a microscope slide on the stage of a microscope. Cover the bead with a drop of the reagent and observe the result through the microscope. A positive test is an active evolution of nitrogen bubbles.

Cut off the portion of the wire covered by the bead of alkali and drop it into a glass capillary of 1-mm. diameter and sealed at one end—e.g., a short melting point tube. Introduce a droplet of the iodine-azide reagent, and swing the tube rapidly, so as centrifugally to project the reagent to the closed end, where it comes into contact with the test bead. While the tube is held with the closed end uppermost, observe whether or not gas is evolved and collected in the upper end.

Tests of procedure 1 using barium sulfate and sodium naphthoquinone sulfonate showed that less than 0.1 microgram of these compounds gave strongly positive results.

2. DETECTION OF SULFUR IN VOLATILE SUBSTANCES. Coat with silver the inner surface of a piece of ordinary (thick-walled) glass tubing of 0.5- to 2-mm. bore by filling with a dilute reagent containing silver nitrate (about 0.1 *N*), ammonium hydroxide, a soluble tartrate, and sodium hydroxide and allowing to stand overnight at room temperature. Empty the tube, wash the interior with distilled water, and dry by warming while a current of air is passed through the tube. Soften one end in a small flame and pull it out to a fine capillary about 2 to 3 mm. long and of 0.1 to 0.2 mm. diameter. Touch the tip of the capillary section to the liquid substance to be tested (if the substance is solid it

may be used molten or dissolved in a sulfur-free solvent); a small quantity of the substance (some tenths of a milligram to several milligrams) will enter. Melt shut the tip of the capillary rapidly by touching it to a hot flame. Apply heat to the tube at a point 2 to 3 cm. from the sealed end, and move the flame gradually toward the sample so as to volatilize it. A slight crackling sound will be heard when the vaporized substance enters the heated zone and decomposes in contact with the silver coating. Allow the tube to cool, break off the tip of the capillary, and allow a droplet of the iodine-azide reagent to enter, observing under the microscope.

The approximate sensitivity limit of this procedure was determined by use of solutions of sulfur in xylene. The tests were performed as "unknowns", the tubes being charged by a coworker. Xylene gave a negative test. With 0.01 microgram of sulfur (0.3 mg. of 0.0033% solution) the result was doubtful. With 0.013 microgram of sulfur (0.4 mg. of 0.0033% solution) the test was positive but not strong, and the same result was noted with 0.02 microgram of sulfur (0.2 mg. of 0.01% solution). Strongly positive tests were observed with sulfur in amounts from 0.05 to 0.12 microgram taken in both concentrations, and a very strong test was given by 0.26 microgram of sulfur (2.6 mg. of 0.01% solution).

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(3) Feigl, Fr., *Z. anal. Chem.*, 74, 369 (1928).
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Final Report on 15-Year Collective Index

The last of the page proof was sent back to the printer on February 12. As this marks the completion of my part in the index, some statistics may not be out of line in this last progress report, as they may be of help in estimating on future indexes.

Subject Index

Job	Started	Completed	Hours
Card-making	March 2, 1944	August 1	354
Editing of cards	August 22	October 13	146
Galley proof came in	December 17	January 8, 1945	
Reading and some checking of galleys	December 17	January 14, 1945	105
Page proof came in	February 8	February 9	
Checking page proof	February 8	February 11	20
		Total	625
Work on Author Index			228
		Grand total hours for C.L.B.	853
Work by Mrs. Bernier, checking galleys			97
Work by Mrs. Swank, making Author Index cards, alphabetizing both indexes, reading and checking galley proof on part of Author Index			275
		Total hours for assistants	372

There are 14,500 cards (entries in the Subject Index) and 2250 cross references. The completed Author Index consists of 6750 cards, including cross references.

As to the quality of the index, we who have worked on it feel it to be high—judging from the few corrections on proof, the fact that about 300 references looked up disclosed no mistakes, and because it was designed with the needs of analytical chemists in mind. Because this index contains more information on methods and reagents, it should prove not only the fastest way, but also the surest way, of getting information out of the first 15 years of the ANALYTICAL EDITION. The fact that the index is built very much like *Chemical Abstracts* indexes makes it possible to turn from one to the other with a minimum of shift in search plan; searchers for organic compound names will especially appreciate this. Mistakes and errors occur in every work of this type; to neutralize these, errata published in the ANALYTICAL EDITION—say once a year—would be a big help. I am, of course, wholly responsible for the correctness of the index and will be glad to help in locating bad references and in straightening out other troubles. I anticipate few mistakes or errors.

I want to express here my appreciation for the skillful work of Mrs. Bernier and Mrs. Swank on this index. We are fortunate in having the help of people with the training and experience of these two.

The counsel of M. G. Mellon and E. J. Crane was invaluable. I wish to record my gratitude not alone for the answers and advice which have so greatly improved the index, but also for the kindness with which these men supplied this information.

CHARLES L. BERNIER

BOOK REVIEW

Introduction to Quantitative Analysis. *Saul B. Arenson* and *George Rieveschl, Jr.* 386 pages. Thomas Y. Crowell Co., 432 Fourth Ave., New York, N. Y., 1944. Price, $2.75.

The book contains chapters on fundamentals, chemical equilibrium, acid-base titrimetry, oxidation-reduction titrimetry, precipitation titrimetry, gravimetric analysis, some applications of electrochemistry, photometric methods, and a small section on the mathematical treatment of analytical results. Scattered throughout the text are 79 laboratory experiments and 291 problems. Decimal points in answers to problems are intentionally omitted, and the problems are apparently taken from actual examples met in the classroom and laboratory.

The presentation of the subject matter is good, although it is occasionally wordy and naively informal, as is exemplified in the following introductory sentence to Overtitration, on page 106: "At the beginning of your careers as volumetric analysts you very seldom 'hit the end point on the head'." In other words, the language of the book strongly reflects the language of the laboratory. Questions which may arise in the minds of critical readers are whether potassium tetraoxalate dihydrate is actually a commonly used standard (p. 96), what constitutes a "volumetric analyst" (p. 106), whether it would not be better to give the students the more recent information on standardizing permanganate (p. 164), whether the decomposition temperature of $BaSO_4$ is actually 800° C. (p. 272), and whether the book gives a reasonably strong picture of 8-hydroxyquinoline (p. 277).

The style of the book conveys the feeling of an unusual frankness between student and teacher. Certain rather lengthy direct quotations from chemical journals may have the good effect of arousing the students' interest in such literature and of overcoming the resistance most students have for reading anything outside their own texts. The explanations of the principles of physical chemistry applicable to analytical chemistry are clear and as simple as possible. Reasonable explanations of simple things are given. For example, the desiccator is defined and its limitations are pointed out, so that the student need not make the mistake of assuming that placing an object in the desiccator affords total protection.

As a text for beginners in analytical chemistry, the book deserves consideration by students and teachers because of the unusual method of presentation.

JAMES I. HOFFMAN