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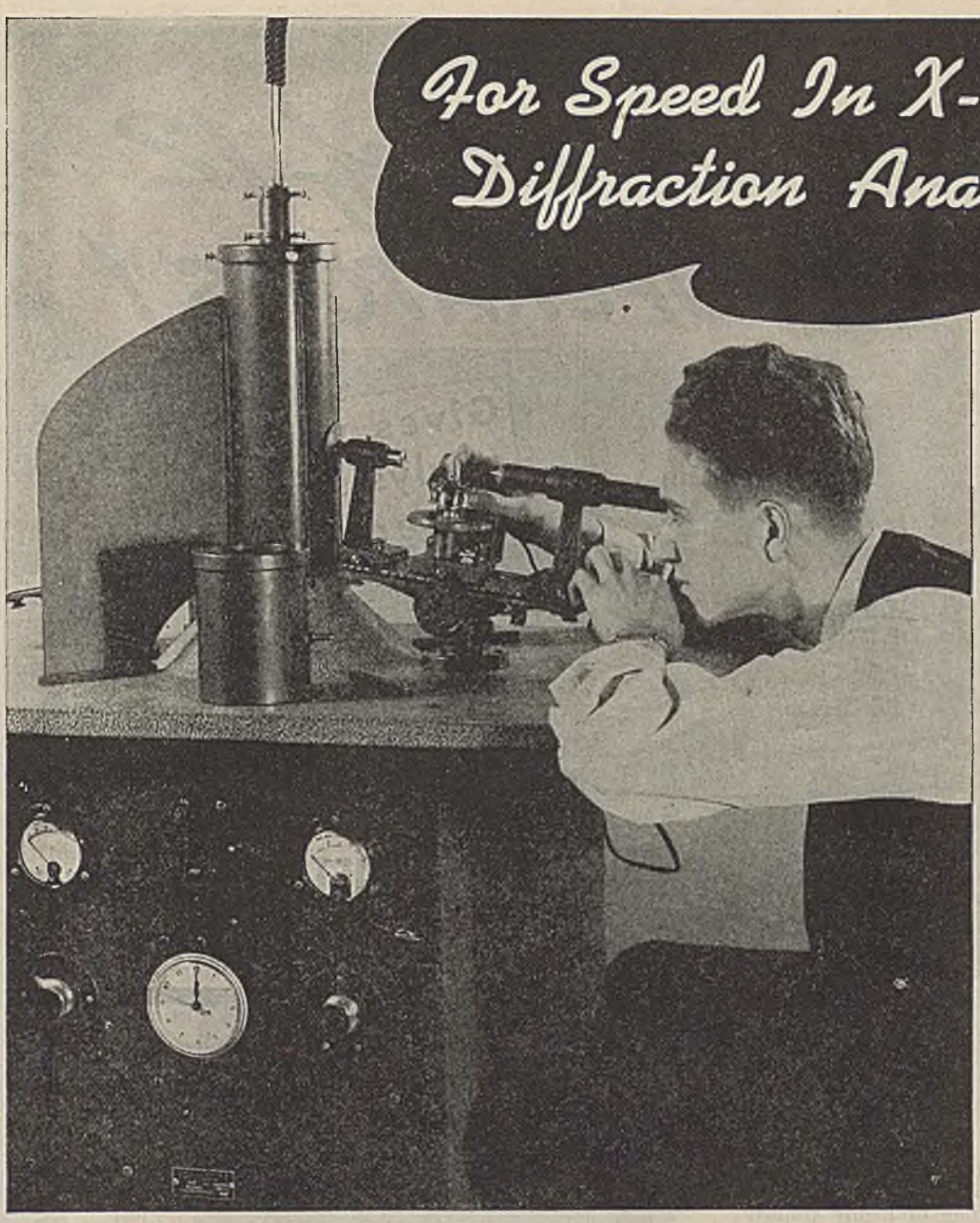

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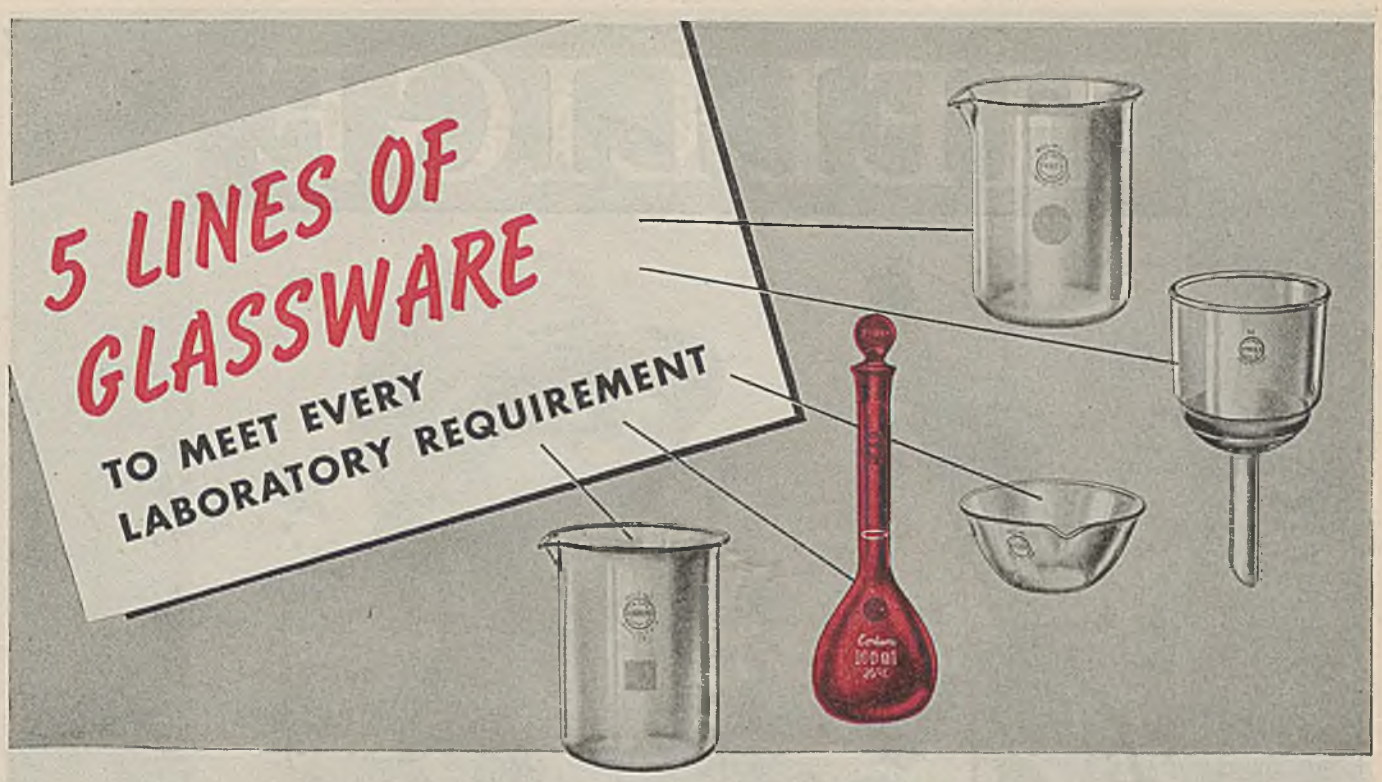

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Number and Type	*Coef. of Expansion*	*Annealing Point*	*Softening Point*	*Strain Point*	*Specific Gravity*	*Loss in Weight—100 lbs. Steam Pressure 96 Hrs. (grams per sq. cm.)*	*Loss in Weight—Boiling 6 Hrs. in 5% NaOH (grams per sq. cm.)*
Pyrex brand Chemical Glass No. 774	0.0000032	560°C	820°C	510°C	2.23	0.0005	0.002
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INDUSTRIAL AND ENGINEERING CHEMISTRY
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Emulsion Polymerization of Synthetic Rubber in 10-Gram Systems

An Experimental Technique

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The experimental procedure consists of sealing the ingredients of a polymerization recipe into a test tube and rotating the tube at a constant temperature, following the course of the reaction by noting the decrease in volume of the system. The latex is removed for coagulation when the polymerization has proceeded as far as desired, and the yield is determined by weighing the dried stabilized coagulum.

IN DEVELOPING practical recipes for the manufacture of synthetic rubber, it was desirable to investigate the effects on the polymerization process of a large variety of highly purified substances. Only small quantities of many materials were available. Furthermore, in order to obtain valid comparisons between experiments conducted over a period of time, it was necessary to keep standardized samples of the major components and to use them as econonomically as possible. These considerations led to the development of a small-scale polymerization technique whereby from 10 to 20 grams of monomers could be employed. By this method an extraordinary amount of valuable information was obtainable. This included:

1. Yield
2. A polymerization reaction curve from which to estimate length of induction period, if any; rate of polymerization at any desired time; and over-all conversion at any desired time
3. Kind of emulsion—i.e., whether fluid, viscous, gelatinous, or heterogeneous, at any stage in the process
4. pH of emulsion at end of process
5. Qualitative observations on coagulation
6. Production of a sample of synthetic rubber sufficiently large to determine solubility, milling characteristics, and cured properties by procedures described by Garvey (*2*)

This small-scale technique has been employed for investigating polymerization of a large number of monomers and comonomer mixtures, for evaluating emulsifying agents, for determining the effect of impurities in the reagents employed, for varying the comonomer ratio and the ratio of hydrocarbons to aqueous phase, and for investigating behavior of various initiators, inhibitors, and other ingredients of the polymerization recipe. It proved to be especially advantageous in providing information on the effect of some one ingredient over a range of concentrations. The influence of reaction temperature was readily determined. In general, the advantages of the technique were particularly apparent in:

Preliminary surveys, where wide areas of investigation had to be covered in the shortest possible time. The method is amenable to simple labor-saving tricks, such as filling a large number of reaction tubes at the same time with solutions of a given emulsifying agent.

Control testing of raw materials. The particular properties essential for polymerization of all shipments of materials intended for large-scale production can be tested quickly. A good correlation can be obtained between the experimental results of this method and behavior on a manufacturing scale.

The most serious limitation to this technique is that materials cannot be added to or subtracted from the system once polymerization has started. [Balandina *et al.* described a similar technique, the details of which are not readily available to English-speaking investigators (*1*).]

RECIPES FOR POLYMERIZING SYNTHETIC RUBBER

The development of satisfactory polymerization recipes is one of the important objectives of synthetic rubber research. Patent literature contains many examples of such. The following, from a U. S. patent issued to Wollthan and Becker (*5*), and recalculated to the scale of this technique, is perhaps typical:

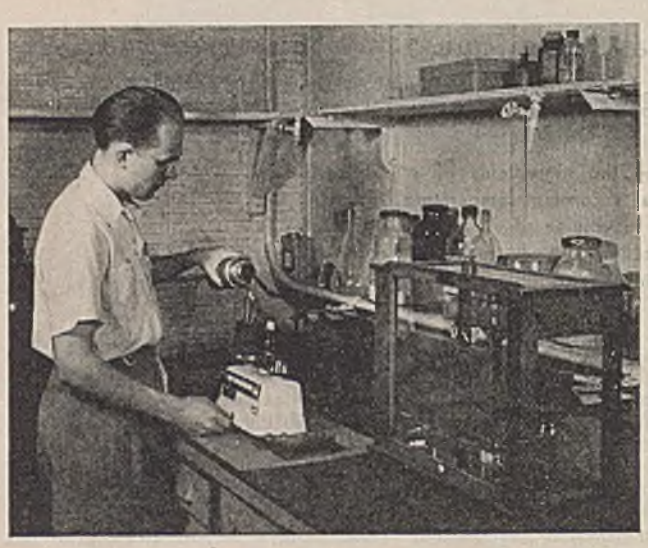
Butadiene	7.5 grams
Styrene	2.5 grams
Isohexyl mercaptan	0.05 gram
Water	18.0 grams
Sodium oleate	2.0 grams
Ammonium persulfate	0.03 gram
Temperature	30° C.
Time	"Several days"
Yield	"Excellent"

An understanding of the function of each ingredient is essential In the above example, the butadiene and styrene are the monomers, which, by copolymerizing, form synthetic rubber. The isohexyl mercaptan is described as a substance exerting a "regulating effect"—i.e., it possibly decreases the branching characteristics of the resulting polymer. Sodium oleate is the emulsifying agent, and the ammonium persulfate acts as the polymerization initiator, also called the "polymerization catalyst".

The possibilities of research on such a system are great and are increased by the fact that a variation introduced in any one ingredient may require a concomitant change in some other ingredient. For example, substituting another substance for the initiator might require a change in the type of emulsifying agent employed in order to get satisfactory results.

GENERAL CONSIDERATIONS ON TECHNIQUE

Polymerization, while a science, is also an art. The way in which things are done—that is, the niceties of experimental technique employed—is of equal importance to the scientific aspects of the subject. Unless this viewpoint is kept clearly in mind, the investigator is frequently confronted by baffling failures. Emulsion polymerization is particularly susceptible to the influence of traces of contaminants. The equipment of a research laboratory may be covered with dust which contains inhibitors or accelerators of polymerization, indeed, some substances, which under certain conditions inhibit polymerization, may under sightly different conditions act as catalysts. Nevertheless, the difficulties confronting the investigator can be avoided with a little care and forethought.



Weighing Butadiene

In general, solutions or other substances to be employed in polymerization experiments should not be exposed to atmospheric contamination for longer periods than necessary. Glass stoppers afford adequate protection. The contents of a flask may be temporarily protected by covering the mouth with a sheet of clean dry tinfoil. Cork stoppers should be avoided; but if necessary, they can be covered with tinfoil. In no case should the alkaline contents of flasks come in contact with tinfoil.

Glass bottles and flasks must be clean. In most cases treatment with chromic acid cleaning solutions, followed by rinsing with tap water and then distilled water, is adequate. Reaction tubes, however, require more effective cleaning.

In one operation it is convenient to pour the volatile contents of a Dewar flask through a short length of rubber tubing. Although rubber contains antioxidants, accelerators, and other chemicals, no trouble is experienced from this source if the rubber tubing is first extracted by boiling in several changes of acetone. This can be done (on a steam plate) in a covered beaker if a sizable piece of dry ice is placed on the watch crystal, which thereby becomes a convenient reflux condenser. The extracted tubing can be kept in a stoppered wide-mouthed bottle for future use.

Certain monomers can be efficiently separated from powerful inhibitors added as stabilizers by distillation through relatively simple equipment. The practice of distilling monomers in the absence of an inhibitor should be avoided because of the danger of explosions due to the accumulation of peroxides in the distillation residue.

It has been customary in the laboratory to prepare fresh samples of butadiene by condensation in clean glassware from a stream of gas taken from a large cylinder of liquefied material. Higher boiling monomers are freshly prepared by atmospheric, vacuum, or steam distillation as required. All-glass distillation equipment is most satisfactory. The unstabilized monomers can be kept stoppered in a refrigerator at −30° C. for several days without detectable deterioration.

Sometimes repetition of an operation is advisable. Metallic polymerization vessels, no matter how carefully cleaned, may be inhibited; merely emptying and recharging are often sufficient to ensure a satisfactory reaction.

There is no substitute for constant care and cleanliness on the part of the investigator. A careful experimenter can easily adjust the weight of small portions of certain monomers using a clean medicine dropper, while a careless experimenter (performing the same operation) can ruin a large number of experiments by allowing the monomer to come into contact with the rubber bulb of the medicine dropper.

EXPERIMENTAL PROCEDURE

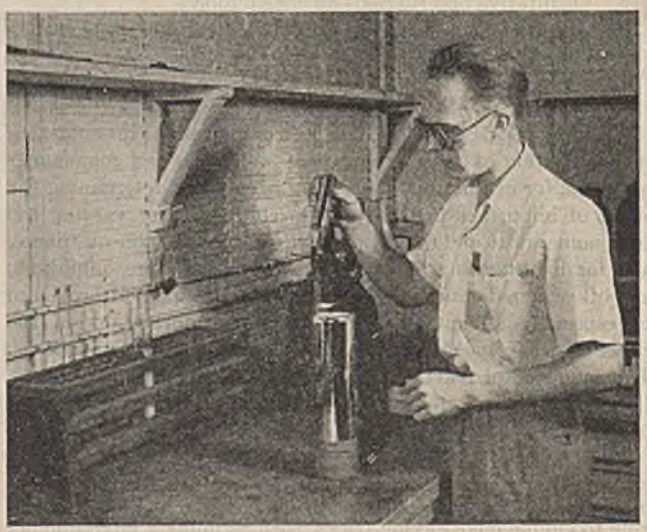
The ingredients of a polymerization recipe are sealed into a test tube, which is rotated at a constant temperature. The course of the reaction is followed by noting the decrease in volume of the system, and the latex is removed for coagulation when the polymerization has proceeded as far as desired. The yield is determined by weighing the dried, stabilized coagulum.

Pyrex reaction tubes, 22 mm. in diameter, approximately 55 ml. in capacity, to the upper end of which are sealed 10-mm. diameter Pyrex tubes, may be obtained in gross lots from the Corning Glass Company, according to the following specifications: "Glass tubes, Pyrex, 22 mm. O.D. × 1.5 mm. walls (uniform), 215-mm. body to neck, 22-mm. tapered shoulder, 145-mm. neck. 10-mm. neck × 1-mm. wall thickness". These can be made by the experimenter, but it has been found cheaper to purchase them.

New tubes are cleaned by rinsing with distilled water and anhydrous C.P. synthetic methanol, in that order, and evacuating until dry. Evacuation may be accomplished with a Cenco Hyvac pump connected in train through a dry ice–acetone trap, which condenses the methanol and prevents diffusion of any volatile inhibitor back into the tube. Acetone-extracted rubber tubing is used to attach the reaction tubes to the vacuum system.

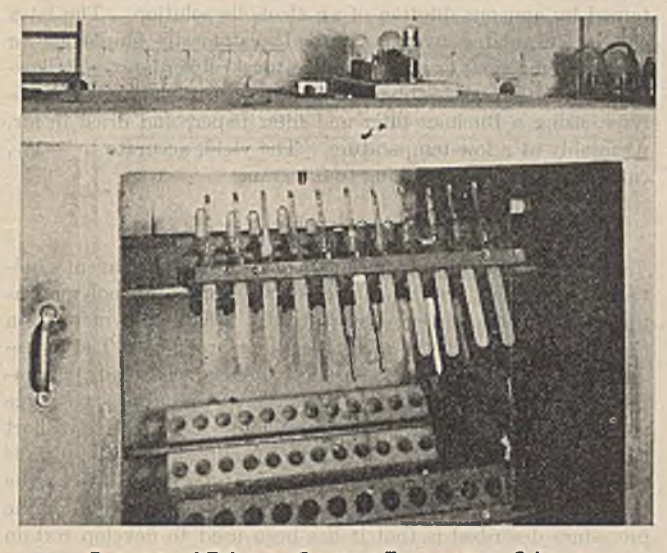
Old tubes, after being cleaned in a chromic acid bath, are repaired by sealing new necks of 10-mm. Pyrex tubing, 10 cm. long, to the shoulder of the tubes. The open ends are fire-polished. The tubes are then subjected to the cleaning treatment recommended by Suess, Pilch, and Rudorfer (4). Clean concentrated nitric acid is poured into the tubes and allowed to stand from 16 to 24 hours. If the tubes are required at once, they are filled to the shoulder with nitric acid and gently heated from 15 to 30 minutes, then rinsed three times with tap water. One rinse should completely fill the tube to displace all the fumes. After two additional rinses with distilled water, the tubes may be dried with synthetic methanol and evacuated. If desired, the methanol rinse and evacuation can be dispensed with by allowing the distilled water to drain from the inverted tubes overnight. The dry tubes are stoppered with No. 0 corks, which have been covered with fresh tinfoil, and the tubes are kept in a clean place until used.

Solids may be added to the reaction tubes in quantities as low as 0.5 mg. from small aluminum foil weighing scoops. If dupli-



Adding Butadiene to Reaction Tube

cate quantities are to be used throughout a series of experiments, it is convenient to dissolve organic compounds in the less volatile comonomer and inorganic compounds in the aqueous solution of emulsifying agent.



Rotation of Tubes in Constant-Temperature Cabinet

The solution of emulsifying agent is generally prepared separately and added to the reaction tubes from a pipet or a graduated cylinder. Since slight variations in the ratio of monomers to aqueous phase have little effect, convenience generally dictates the use of the latter method. The volume of emulsifying agent employed may vary from 10 to 30 ml. for 10 grams of monomers.

The reaction tubes containing the emulsifying agent and other ingredients are placed in a refrigerator, inclined at an angle of 2° 8′ from horizontal, and frozen at −30° C. This inclination may be obtained by placing a piece of 10-mm. glass tubing under the necks of the tubes. Unless chilled in this position, the tubes will crack when placed in a dry ice–acetone bath. (The cork-stoppered glass reaction tubes may be laid on a table at the proper inclination and covered with dry ice. However, any contamination of the contents by carbon dioxide will alter the pH of the soap solution and affect the reaction rate seriously.) If care is exercised, the aqueous phase can be frozen by direct immersion of the tubes in a dry ice–acetone bath, with frequent withdrawals and nearly horizontal rotations, so that the aqueous phase freezes in contact with the glass in the form of a hollow cylinder. The losses due to cracking of the glass are high and the operation is time consuming; therefore the method of freezing first described is preferred in most cases.

In the next step the frozen tubes are individually cooled further in a dry ice–acetone mixture contained in a quart-size straight-sided Dewar flask. A rubber dam is fitted over the tube and the Dewar flask, the neck of the tube extending through a hole in the dam. This minimizes contamination of the tube with escaping carbon dioxide, and holds it in a convenient vertical position.

The higher boiling comonomer is weighed to 0.1 gram into a tared, clean microbeaker and poured into the reaction tube. A buret is sometimes used for this operation but contamination by stopcock grease should be avoided. In either case the operation must be performed in a rigorously clean manner.

Freshly distilled butadiene is temporarily contained in a pint-size Dewar fitted with a two-hole extracted rubber stopper through which extend two short glass tubes arranged for convenient pouring. The temperature of the butadiene is held at −30° C. It is weighed to 0.1 gram into a small silvered Dewar weighing flask using a torsion balance. The small Dewar is then attached to the reaction tube by a short length of extracted rubber tubing and the butadiene is poured into the reaction tube, allowing about 45 seconds for condensation of vapor before removing the rubber tube. The weighing flask has a 10-mm. neck, and is 11.5 cm. from bottom to shoulder, 35 mm. in outside diameter, 25 mm. in inside diameter, and about 16 cm. in over-all length. Such flasks have been made in the laboratory but it has been found more satisfactory to have them made by professional glass blowers.

The reaction tube is sealed, using a hand blow torch. It is advisable to do the sealing close to the open end of the neck in an oxidizing atmosphere; otherwise a carbon mirror may form on the interior surface of the tubing and prevent a tight seal. Occasionally, if condensation is not complete, a slow blue flame travels from the heated glass down into the reaction tube. It has been impossible, however, to demonstrate that this brings about any variation in the ensuing polymerization.

POLYMERIZATION

The sealed reaction tube is brought to reaction temperature by immersion in water. The height of the meniscus is determined and recorded, using a millimeter rule. The tube is then rotated at a constant temperature and readings of the meniscus height are made periodically. It is from these readings that reaction curves such as Figure 1 can be plotted.

If the polymerization is complete in less than 5 hours, a water thermostat is required to prevent temperature buildup. However, a thermostatically controlled air cabinet, provided with shafts for rotating the tubes, is more convenient.

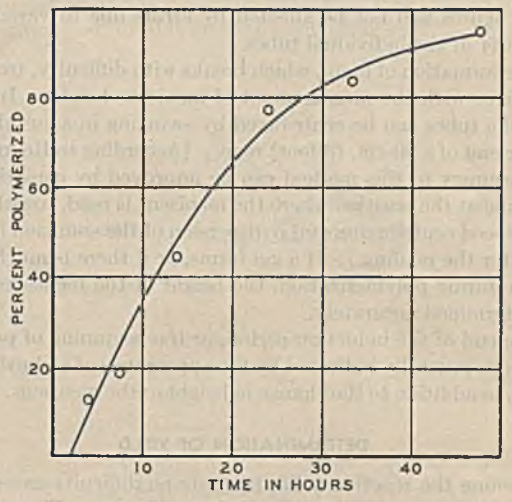
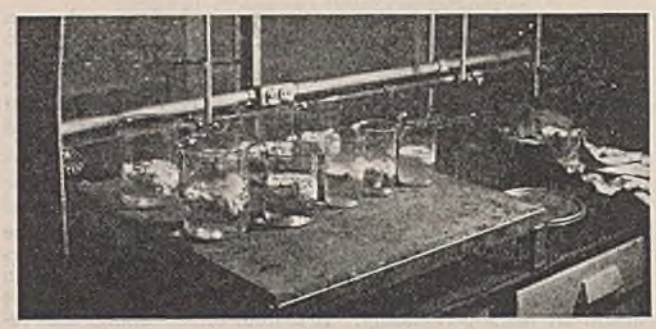


Figure 1. Polymerization Curve

A reaction tube, prepared as described, using the Wollthan and Becker recipe (*5*), will give an initial meniscus height of approximately 110 mm. During the course of polymerization, it will drop 11 mm. Since the height can be read to 0.5 mm., this procedure provides a simple method of following the reaction rate with an accuracy of about 5%. Table I shows for other recipes that the drop in height of the meniscus is directly proportional to the yield of polymer, within the limits of error of the method. If the total decrease of meniscus height is measured just before opening the tube, the yield, as measured on the dry polymer, will

Table I. Correlation between Percentage Polymerized and Fall of Meniscus for Three Polymerization Recipes

	Fall of Meniscus	Yield		
		Measured	Calculated from meniscus	Difference
	Mm.	%	%	%
Series I	3.0	18	19	+1
	7.0	39	44	+5
	10.4	61	65	+4
	14.1	89	89	...
Series II	3.0	21	19	−2
	7.0	43	45	+2
	10.9	70	70	0
	14.4	93	93	...
Series III	2.0	9	13	+4
	4.0	20	26	+6
	4.5	26	29	+3
	5.8	38	37	−1
	7.6	44	49	+5
	7.8	47	50	+3
	11.0	67	70	+3
	12.6	86	81	−5
	14.7	94	94	...



Washing Coagulated Synthetic Rubber Samples

give an accurate basis for calculating the partial yields at any given time during the process. Reaction curves plotted using these figures will not be affected by errors due to variations in diameter of the individual tubes.

The formation of foam, which breaks with difficulty, frequently interferes with the measurement of meniscus height. In such a case the tubes can be centrifuged by swinging in a suitable tube on the end of a 90-cm. (3-foot) rope. [According to Reynolds (*3*) the accuracy of this method can be improved by constriction of the tube at the position where the meniscus is read, together with high-speed centrifuging and redispersion of the emulsion by shaking after the reading.] If a gel forms, or if there is much coagulation during polymerization, the height of the meniscus cannot be determined accurately.

The end of the induction period, or the beginning of polymerization, is generally indicated by the appearance of a bluish opalescence, in addition to the change in height of the meniscus.

DETERMINATION OF YIELD

Opening the reaction tubes presents no difficulty except when low partial conversions are under investigation. Then it is necessary to break the tip and direct the violently expelled foam into a large beaker. The synthetic rubber latex must next be stabilized by the addition of an antioxidant. Two per cent of dispersed phenyl-beta-naphthylamine has been found convenient and satisfactory. The dispersion of the stabilizer can be obtained by aqueous dilution of an alcoholic solution. The latex can be coagulated by any method customarily employed for breaking emulsions or coagulating natural rubber latex. Following coagulation, the rubber is washed free of soap and electrolytes, using a Büchner filter and filter paper, and dried in air, preferably at a low temperature. The yield, accurate to ±2%, can be obtained by weighing to 0.1 gram.

SUMMARY

Many manufacturers of monomers, emulsifying agents, initiators, modifiers, and other ingredients going into polymerization reactions find it necessary to have a reliable polymerization procedure for testing the quality of their products. The procedure presented, despite some shortcomings, has many advantages. Minimum amounts of material are required and large numbers of experiments can be conducted in a relatively short time. A serious effort is made to point out some of the pitfalls which beset investigators of polymerization regardless of the type of technique employed. The best recommendation for the procedure described is that it has been used to develop certain types of synthetic rubber which are now in commercial production.

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Presented before the Division of Rubber Chemistry at the 105th Meeting of the American Chemical Society, Detroit, Mich.

Determination of Tetraethyllead in Gasoline

HARRY GONICK AND J. J. MILANO, Shell Oil Company, Incorporated, Martinez, Calif.

A method for the determination of tetraethyllead in gasoline is described in which the tetraethyllead is decomposed with iodine and the lead subsequently titrated by a new acidimetric method employing 8-hydroxyquinoline. The method is rapid and is applicable to all types of gasolines.

UNTIL recently the most widely used method for the determination of tetraethyllead in gasoline was the bromination method described by Edgar and Calingaert (*3*) in which the tetraethyllead was decomposed by the action of bromine. Although this method was rapid and convenient for the determination of tetraethyllead in straight-run gasolines, difficulties were encountered with cracked gasolines owing to the rapid absorption of bromine by the olefins present, in competition with the tetraethyllead. With gasolines of high olefin content it was necessary to brominate the gasoline completely to ensure complete decomposition of the tetraethyllead. Even so, low results were frequently obtained. Moreover, the quantity of bromine required for complete bromination of a gasoline of high olefin content was rather large (frequently in excess of 200 grams) and added substantially to the cost of the analysis. In addition, the bromination reaction was violent and was accompanied by the evolution of corrosive vapors which caused considerable hazard to the operator. For these reasons, the bromination method was not suited to the routine analysis of cracked fuels.

More recently other methods have been devised in which the gasoline is treated with hydrochloric acid and the lead determined in the acid extracts. The best known of these is the method of Calingaert and Gambrill (*2*), recently adopted by the American Society for Testing Materials as a tentative standard (*1*). Although this method gives satisfactory results with all types of gasolines, the over-all time required for a determination is somewhat lengthy and specialized equipment is required.

The method described in this paper was developed in an effort to reduce the time required for the tetraethyllead determination. By the proposed method, a single determination can be completed in one hour and for determinations in quantity only a small fraction of this time is required per determination. The accuracy of the method appears to be equal to previous methods and does not appear to be affected by the type or composition of the gasoline. More than three thousand samples of gasoline,

representing all the principal brands and grades sold in the western states, have been analyzed successfully.

PRINCIPLE OF PROPOSED METHOD

As in previous methods, the determination of the tetraethyllead divides itself into two distinct parts: (1) the decomposition of the tetraethyllead to yield an inorganic lead salt, and (2) the determination of the lead.

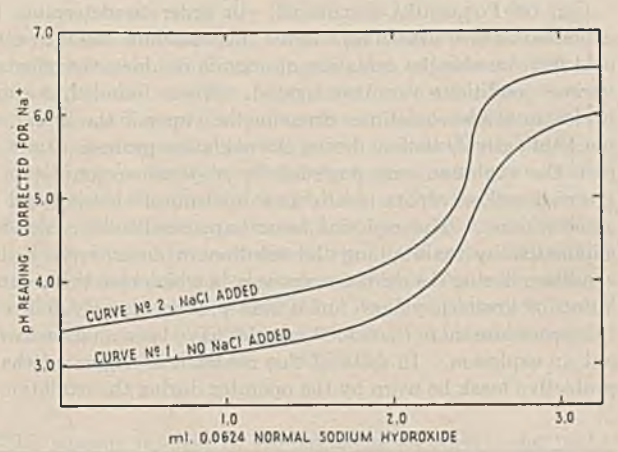

Figure 1. Effect of Sodium Chloride on Neutralization Curve of Solutions Containing Lead Ions

0.137 gram of $[Pb(NO_3)_2$ in 100 ml. of water. (1) No NaCl added, (2) 5 grams of NaCl added

1. Experiments conducted in this laboratory showed that tetraethyllead is rapidly and quantitatively decomposed by the action of free iodine. Unsaturated hydrocarbons do not interfere, as they do not iodinate so rapidly as to compete with the tetraethyllead reaction. The gasoline is removed by evaporation under a hot air stream, and any organic matter remaining is subsequently oxidized with nitric acid and potassium chlorate. The inorganic residue remaining then contains all the lead in the form of inorganic salts including lead iodate, which is insoluble in water. The lead salts so obtained are converted to the more soluble chloride by treatment with hydrochloric acid.

2. The lead is determined by an acidimetric titration method; it is therefore necessary to neutralize the solution exactly before titrating the lead. The neutralization of a solution containing lead salts ordinarily presents difficulties owing to the hydrolysis of the lead. These difficulties are obviated, however, by the presence of sufficient chloride ions which effectively suppress the hydrolysis of the lead. The effect of sodium chloride on the neutralization of a lead solution is shown by the curves in Figure 1.

After the solution has been neutralized, an excess of 8-hydroxyquinoline is added. This reagent reacts with the lead ions to liberate an equivalent quantity of acid which is then titrated with standard alkali. The reactions are assumed to be as follows:

$$HOC_9H_6N = {}^-OC_9H_6NH^+ \quad (1)$$

$$Pb^{++} + 2{}^-OC_9H_6NH^+ = Pb(OC_9H_6NH^+)_2 \quad (2)$$

$$Pb(OC_9H_6NH^+)_2 + 2OH^- = Pb(OC_9H_6N)_2 + 2H_2O \quad (3)$$

As indicated in Equation 1, 8-hydroxyquinoline is amphoteric and goes over to the ionic form. When 8-hydroxyquinoline reagent is added to a solution containing lead ions, lead 8-hydroxyquinolinium ions are formed according to Equation 2. These are quantitatively titrated with standard alkali to pH 7 according to Equation 3. Other equilibrium reactions are undoubtedly involved, including reaction between lead 8-hydroxyquinolinium ions and excess reagent; however, the equations given indicate the essential result.

DETAILS OF METHOD

Apparatus. The hot air–jet evaporator (Figure 2) is designed to direct a hot air stream into four Erlenmeyer flasks simultaneously during evaporations. Although the design shown has proved very satisfactory in actual practice, other designs which will accomplish the same purpose may be used.

Reagents. Iodine, saturated solution in carbon tetrachloride (technical). Nitric acid, c.p., concentrated. Potassium chlorate, c.p., crystals. Hydrochloric acid, c.p., dilute solution; 1 to 1. Sodium chloride, c.p., crystals. 8-Hydroxyquinoline, 0.065*N* in 60 per cent isopropyl alcohol. Standard sodium hydroxide, 0.0624*N*. Standard hydrochloric acid, 0.0624*N*. (Standard 0.0624*N* acid and base were selected since these reagents are in general use in oil laboratories.)

Methyl red indicator; dissolve 1 gram in 600 ml. of alcohol and dilute to 1 liter with water. Phenol red indicator, 0.2 gram per liter of water.

Separation of Lead. Measure exactly 100 ml. (corrected to 60° F.) of the gasoline to be tested into a 500-ml. Erlenmeyer flask.

Add 50 ml. of the iodine solution and allow to stand for at least 5 minutes. Place the flask on a hot plate under a hot air stream and evaporate the gasoline to dryness. The velocity of the air stream and the temperature of the hot plate should be regulated so as to secure the maximum rate of evaporation without spattering or bumping. The air stream effectively suppresses the tendency toward bumping which is almost unavoidable without its use. The evaporation ordinarily takes from 15 to 20 minutes.

Add 25 to 50 ml. of concentrated nitric acid (depending on the amount of the organic residue) and rotate the flask over a burner until dense fumes of iodine and nitrogen dioxide cease. Should any organic matter adhere to the walls of the flask continue rotating the flask until it is completely dislodged.

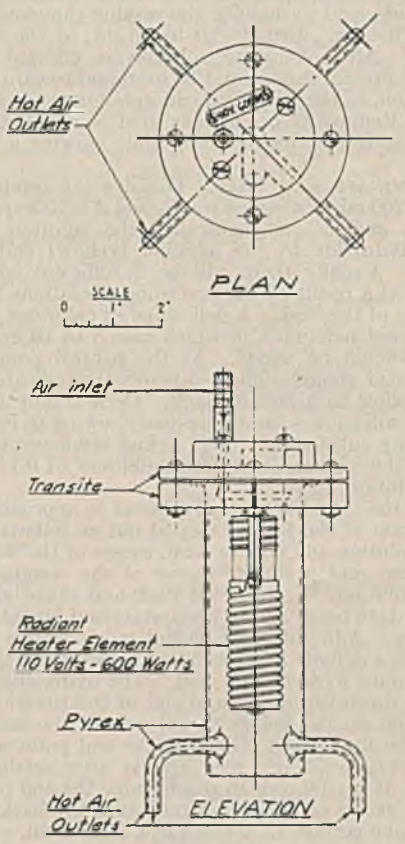

Figure 2. Hot Air–Jet Evaporator

All interior metal parts must be heat-resistant

To the actively boiling solution add crystals of potassium chlorate until the organic matter is completely destroyed. The potassium chlorate should be added cautiously. The solution should not be permitted to evaporate to dryness while there is visible organic matter present as indicated by a brownish coloration of the solution; otherwise spontaneous ignition will occur and cause losses of lead. Usually all organic matter disappears after the first 2 or 3 grams of potassium chlorate have been added; however, one or two additional portions of 2 or 3 grams each are added in order to complete the oxidation. With practice, the oxidation of residues from cracked gasolines with potassium chlorate can be effected in 1 to 3 minutes.

Evaporate the clear nitric acid solution to complete dryness. Should the solution exhibit any darkening during the evaporation,

Table I. Determination of Tetraethyllead in Synthetic Gasoline Blends by the Proposed Method[a]

Samples	Tetraethyllead Content Calculated	Determined
	Ml./gallon	
80% cracked gasoline + 20% straight-run gasoline	3.07	3.07, 3.06, 3.07
	1.54	1.54, 1.54, 1.56
	0.20	0.19, 0.20, 0.19
100% straight-run gasoline	3.00	3.00, 3.00
	1.50	1.51, 1.50
	0.30	0.29, 0.29
50% cracked gasoline + 50% isopentane	1.54	1.54
50% cracked gasoline + 50% alcohol	1.54	1.55

[a] These blends were made using ethyl fluid obtained from the Ethyl Corp.

add more potassium chlorate. The use of the hot air stream is not recommended during this operation, as the cooling effect of the air stream impedes the oxidation of possible traces of organic matter. To ensure the complete destruction of organic matter, heat the residue over a burner until it is completely fused. The residue after fusion should be white (see note below on the use of potassium chlorate).

Allow the flask to cool somewhat, and add sufficient 1 to 1 hydrochloric acid to dissolve the residue completely after 2 or 3 minutes' boiling. Usually 10 to 20 ml. of the dilute acid are sufficient. After complete solution is effected evaporate to dryness. Special care should be exercised toward the end of the evaporation, as the potassium chloride formed has a tendency to spatter. Remove the remaining acid as completely as possible by thoroughly heating the flask while blowing a hot air stream into it.

Determination of Lead. Dissolve the residue in the flask in 150 to 200 ml. of distilled water, add 2 or 3 drops of methyl red indicator, and exactly neutralize the solution with 0.0624*N* sodium hydroxide to the alkaline (yellow) end point of the indicator. Usually there will be a sufficient concentration of chlorides as a result of the preceding operations to suppress the hydrolysis of the lead. A deficiency of chlorides will render the neutral point indefinite, in which case 5 to 10 grams of sodium chloride should be added. At the neutral point one drop of 0.0624*N* acid should suffice to revert the indicator color from a canary yellow to a definite pink. Occasionally the methyl red indicator will show a fading tendency, owing to remaining traces of oxidizing substances. This fading tendency is readily overcome by the addition of a few milliliters of 0.1*N* sodium thiosulfate solution.

Where the quantity of lead present is approximately known, the titration of the lead is carried out as follows: To the neutralized solution add a 2- to 3-ml. excess of the 8-hydroxyquinoline reagent and a similar excess of the standard sodium hydroxide solution. Stopper the flask and shake vigorously for a few seconds to break up the precipitate and liberate any occluded substances. Add sufficient phenol red indicator (about 3 ml.) to produce a definite pink color and back-titrate the excess alkali with standard hydrochloric acid. The hydrochloric acid should be added dropwise toward the end of the titration and the end point taken on the yellow (acid) side of the indicator change. Agitate the flask when observing the end point and ignore any pink fluorescence which may appear after settling of the precipitate. It is advisable to redetermine the end point by adding a further excess of alkali and repeating the back-titration with acid. Make certain that there is a 2- to 3-ml. excess of the 8-hydroxyquinoline reagent over the net volume of alkali consumed.

When the approximate quantity of lead is not previously known, the titration must be carried out stepwise in order to secure the correct excess (2 to 3 ml.) of the 8-hydroxyquinoline reagent. Add about 3 ml. of phenol red indicator to the solution which has been previously neutralized to methyl red. Now add the 8-hydroxyquinoline in 3-ml. increments and after each addition add an equal volume of 0.0624*N* sodium hydroxide. An excess of the 8-hydroxyquinoline reagent is indicated when the addition of the alkali increment renders the solution alkaline (pink) to the phenol red indicator. At this point stopper the flask, shake vigorously for a few seconds, and back-titrate the excess alkali with standard acid to determine the approximate consumption of alkali. Adjust the volume of 8-hydroxyquinoline added, so that there is an excess of 2 to 3 ml. over the net volume of alkali consumed (volume of standard alkali minus volume of standard acid). A larger excess of 8-hydroxyquinoline should be avoided, as this reagent exhibits a slight buffering effect which interferes with the end-point determination. Redetermine the end point after the addition of a further 2 to 3 ml. of standard alkali by back-titration with standard acid as already described.

Two equivalents of titratable acid are formed for each mole of lead. The solutions should be standardized against a known quantity of lead, using the same titration procedure as in the analysis. Pure test lead or lead nitrate may be used as a standard.

The result expressed in milliliters of tetraethyllead per gallon of gasoline is obtained by multiplying the PbO equivalent (expressed in grams) of the net volume of sodium hydroxide consumed by 33.24.

Use of Potassium Chlorate. In order to determine the explosion hazard attending the use of potassium chlorate–nitric acid mixtures for the oxidation of organic residues, the effects of various conditions were investigated. It was found that a mild explosion would sometimes occur in the vapor if the liquid was not kept actively boiling during the oxidation process. In every case the explosion was preceded by a dense accumulation of greenish yellow vapors (probably a mixture of chlorine and organic vapors). The explosion hazard appeared to be completely eliminated by maintaining the solution in an actively boiling condition during the oxidation process, in which case the accumulation of greenish yellow fumes was prevented. By following this procedure more than 3000 samples have been analyzed without an explosion. In spite of this record it is suggested that a protective mask be worn by the operator during the oxidation.

Table II. Comparison of A.S.T.M. and Proposed Methods

Samples	Tetraethyllead Content A.S.T.M. method	Proposed method
	Ml./gallon	
Competitive Q gasolines		
Brand I	0.26	0.26
Brand II	0.06	0.07
Brand III	1.26	1.25
Brand IV	0.37	0.38
Brand V	0.53	0.53
Competitive Ethyl gasolines		
Brand I	1.35	1.35
Brand II	1.78	1.80
Brand III	1.92	1.93, 1.94, 1.93
Brand IV	1.87	1.87
Brand V	1.58	1.57
Brand VI	2.01	2.01
Brand VII	1.06	1.08
Aviation gasolines		
Brand I	3.04	3.04
Brand II	3.05	3.06
Brand III	2.97, 2.97, 2.97	3.00, 3.00, 2.99

ACCURACY OF THE METHOD

A series of synthetic blends of tetraethyllead in cracked and straight-run gasolines was prepared to check the accuracy of the method. Blends were also prepared with the addition of isopentane and alcohol and analyzed by the described method. The results of these experiments are shown in Table I.

A comparison of results obtained by the proposed method and by the A.S.T.M. method (*1*) is shown in Table II.

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American Society for Testing Materials Meetings

The 1944 Spring Meeting and Committee Week of A.S.T.M. is to be held in Cincinnati, Ohio, at the Netherland Plaza from February 28 to March 3. The 47th Annual Meeting will be held in New York, N. Y., at the Waldorf-Astoria June 26 to 30, 1944.

Specific Gravity of Butadiene

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The specific gravities (t/60° F.) of 1,3-butadiene of 99.6 mole per cent purity were determined experimentally for the temperature range of −17.78° to 60° C. (0° to 140° F.), by means of a specially constructed steel pycnometer of approximately 4500-cc. capacity and capable of withstanding the resultant vapor pressures without undergoing a permanent change in volume. The experimentally determined specific gravities were smoothed graphically and the experimental and smoothed values were compared to show the magnitude of the probable error in the smoothed data. The densities reported here were compared with densities found in the literature, and it was concluded that the final interpolated values determined in this work were probably correct to ±0.00015. The best value for the specific gravity (60°/60° F.) of pure 1,3-butadiene was estimated to be 0.6274 ± 0.00015.

THE current interest in 1,3-butadiene as a synthetic rubber raw material has created a need for more accurate and more complete information on the physical properties of this hydrocarbon.

Landolt and Börnstein (*2*) give a table of liquid densities for 1,3-butadiene for the temperature range −20° to 20° C. (−4° to 68° F.), Prevost (*4*) has reported a value at 6.22° C. (21.2° F.), and Doss (*1*) lists a value at 68° F. Because these data cover a range of temperature too small to meet most requirements, it seemed advisable to check previous values and extend the temperature range. This paper reports experimentally determined liquid specific gravities for 1,3-butadiene for the temperature range −17.78° to 20° C. (0° to 140° F.).

METHOD

The procedure consisted essentially of comparing the determined weights of known volumes of butadiene under a number of temperature conditions and under pressures approximately equal to the vapor pressures with the weights of identical volumes of water at 15.56° C. (60° F.) and at atmospheric pressure. This procedure has been employed previously by the writers to arrive at similar data for propane, iso- and *n*-butane, the various butylenes, and a number of commercial products falling in the classification of liquefied petroleum gases (*3*).

COMPOSITION OF BUTADIENE USED

The 1,3-butadiene used in the investigation was obtained from the by-product butadiene plant of the Phillips Petroleum Company and was representative of the commercial product of the plant at the time of sampling. The sample was inhibited against oxidation with 0.02 weight per cent of phenyl-beta-naphthylamine. No solvent for the inhibitor was used. The quantity of added inhibitor was calculated to increase the specific gravity by no more than 0.00005 and its presence in the sample could, therefore, be ignored.

The composition of the sample was ascertained by two different analytical techniques, both based upon the well-known chemical reaction between maleic anhydride and 1,3-butadiene. Analyses by the two techniques gave a purity of 99.6 mole per cent. The impurities present were believed to consist of 1-butene and the high- and low-boiling 2-butenes.

APPARATUS

The apparatus consisted of two steel pycnometers of approximately 4500-cc. capacity fitted with expansion chambers to facilitate measurements at temperatures below room temperature. A constant-temperature bath, a centrifugal pump for stirring the bath liquid, a torsion balance with calibrated weights, and a calibrated mercury-in-glass thermometer were also provided.

The details of one of the pycnometer units are shown in Figure 1, where *A* is the pycnometer and *B* is the expansion chamber. *C* is a high-pressure stainless steel needle valve of such construction that when fully opened the pressure of the material in the chambers is held by a metal-to-metal seat instead of by valve packing, thus reducing the chance for errors due to leakage. Valve *D* is a brass body steel needle valve. Both pycnometer units were tested before use with hydrogen gas at 27-kg. (600 pounds) pressure to assure absolute freedom from leaks. The two units were used simultaneously for check determinations.

The thermometer used for the measurement of bath temperatures was graduated in 0.2° F. divisions. It was checked before use against a Bureau of Standards calibrated mercury-in-glass thermometer.

The torsion balance was checked before use for accuracy, sensitivity, stability, and equality of length of balance arms. It was tested during use for reproducibility of weights by weighing an iron weight of about 9 kg. (20 pounds) at various times during the day and on successive days. These tests indicated that a weight in the desired range—i.e., 7.7 to 8.6 kg. (17 to 19 pounds)—could be reproduced to 68 mg. (±0.0015 pound). The set of brass weights used were calibrated to 9 mg. (±0.0002 pound).

CALIBRATION OF PYCNOMETERS. After being carefully cleaned and dried, both internally and externally, the two pycnometer units were evacuated and the tare weights determined and checked by repeated weighings to the nearest 22 mg. (0.0005 pound). The volumes of the pycnometer chambers were then ascertained for temperatures of 4.44°, 15.56°, 26.67°, 37.78°, 48.89° and 60° C. (40°, 60°, 80°, 100°, 120°, and 140° F.), and with no internal pressure on the chambers, by weighing the water-filled chambers at the various temperatures and then making corrections for the changing density of water. Freshly boiled distilled water was used. The effect of internal pressure on pycnometer chamber volumes was ascertained for a temperature condition of 15.56° C. (60° F.) and pressures of 0, 14, and 28 kg. per sq. cm. (0, 200, and 400 pounds per square inch) gage, respectively. The final results of the calibrations expressed in terms of volume for various conditions of temperature and internal pressure, were plotted to arrive at a smooth relationship for use in the subsequent experiments. It is believed that the finally assigned volumes for the various conditions were known to ±0.2 cc. for the entire temperature range.

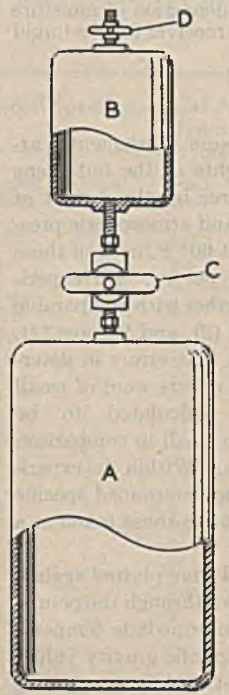

Figure 1. Metal Pycnometer Unit

MEASUREMENT OF DENSITIES

The pycnometer units were evacuated to an absolute pressure of less than 1 mm. of mercury. Sufficient butadiene was then charged into the units to fill *A* completely and *B* to half its capacity. Valve *D* was closed and *C* was left open. The units were then placed in a constant-temperature bath in such a manner that cell *A* was totally immersed in the bath liquid, but with no part of cell *B* immersed. Heat was applied externally to *B* by means of an electrically heated removable jacket to maintain its temperature, 6° to 9° C. (10° to 15° F.) higher than the bath temperature. The temperature of *B* was measured by a thermocouple on the outside surface of the cell at a point below the liquid level in the cell. In a preliminary series of observations it was determined that approximately one hour was required to bring the temperature of the pycnometer and its charge of butadiene to the bath temperature. The pycnometers were consequently held in the constant temperature bath for 1.5 hours before being

Table I. Summary of Experimentally Determined Specific Gravities of 1,3-Butadiene

This Work (99.6 Mole Per Cent 1,3-Butadiene)

Temperature ° F.	Pycnometer No.	Specific gravity ($t/60°$ F.)[a]	Literature, Specific Gravity ($t/60°$ F.)[a]
0	1	0.66671	0.6659 (2)[b]
	1	0.66671	
	2	0.66675	
	2	0.66675	
20	1	0.65414	0.6640 (2)[b]
	2	0.65410	
21.2			0.6493 (4)
40	1	0.64097	0.6409 (2)[b]
	1	0.64097	
	2	0.64070	
	2	0.64065	
60	1	0.62732	0.6273 (2)[b]
	2	0.62725	
68			0.6213 (1)
80	1	0.61361	
	2	0.61371	....
100	1	0.59933	....
	1	0.59944	
	1	0.59944	
	2	0.59932	
	2	0.59937	
	2	0.59947	
			....
120	1	0.58445	
	2	0.58422	
140	1	0.56920	....
	1	0.56890	
	2	0.56850	
	2	0.56870	

[a] Specific gravity at temperature t with reference to water at 60° F.
[b] Interpolated values.

Table II. Smoothed Specific Gravity Values for Commercially Pure 1,3-Butadiene

(99.6 mole per cent 1,3-butadiene)

Temperature ° F.	Specific Gravity ($t/60°$ F.)[a]	Temperature ° F.	Specific Gravity ($t/60°$ F.)[a]
0	0.6668	72	0.6191
2	0.6655	74	0.6177
4	0.6643	76	0.6163
6	0.6631	78	0.6149
8	0.6618	80	0.6135
10	0.6606	82	0.6121
12	0.6593	84	0.6106
14	0.6580	86	0.6091
16	0.6567	88	0.6077
18	0.6554	90	0.6063
20	0.6541	92	0.6049
22	0.6529	94	0.6035
24	0.6515	96	0.6021
26	0.6502	98	0.6007
28	0.6489	100	0.5993
30	0.6476	102	0.5978
32	0.6463	104	0.5963
34	0.6449	106	0.5949
36	0.6436	108	0.5934
38	0.6423	110	0.5919
40	0.6409	112	0.5904
42	0.6396	114	0.5889
44	0.6382	116	0.5874
46	0.6369	118	0.5859
48	0.6356	120	0.5844
50	0.6342	122	0.5828
52	0.6328	124	0.5813
54	0.6314	126	0.5798
56	0.6300	128	0.5783
58	0.6286	130	0.5767
60	0.6273[b]	132	0.5751
62	0.6259	134	0.5736
64	0.6245	136	0.5720
66	0.6232	138	0.5704
68	0.6218	140	0.5689
70	0.6205		

[a] Specific gravity at temperature t with reference to water at 60° F.
[b] Probable specific gravity (60°/60° F.) for pure 1,3-butadiene, arrived at by applying corrections for assumed impurities, was estimated to be 0.6274 ± 0.00015.

removed for weighing. During that time, the bath temperature was held constant to 0.06° C. (±0.1° F.). Just before removing a pycnometer from the bath, C was closed. Immediately upon removal, the outside surface was dried and B was evacuated. D was then closed, C was opened, and the unit was weighed. In those instances where the bath temperature was below the dew point temperature of the room air, the temperature of the pycnometer was raised to above room temperatures to avoid errors in weighing occasioned by condensation of moisture on the surface. In such cases B served as a receiver for the liquid butadiene displaced from A.

RESULTS

The specific gravities of the butadiene were subsequently arrived at by dividing the determined weights of the butadiene contained in A at the various temperatures by the weight of the same volume of water when at 60° F. and atmospheric pressure. The value for the density of water at 60° F. used in these calculations was taken as 0.999017 gram per cc. All experimental results are presented in Table I together with comparable data from Doss (*1*), Landolt and Börnstein (*2*), and Prevost (*4*).

An analysis of the method used showed that errors in determined gravities traceable to air buoyancy effects were of small magnitude. The buoyancy correction, calculated to be +0.00003, was not applied since it was too small in comparison with the experimental error to be significant. Within the experimental error it has been concluded that the determined specific gravity values can be accepted as equivalent to those taken in a vacuum.

The specific gravities presented in Table I were plotted against temperature, and, from a smooth curve drawn through the points, the specific gravity values for the various intermediate temperatures were determined. These smoothed specific gravity values are presented in Table II. Of the twenty-two different specific gravity measurements made at temperatures of 48.89° C. (120° F.) and below, only those made at 4.44° and 26.67° C. (40° and 80° F.) in pycnometer unit 2 differed from the smoothed value by more than the predicted probable amount of 0.00018.

It was believed that the 0.4 mole per cent of impurities present in the sample consisted of 1-butene and the high- and low-boiling 2-butenes. By making certain assumptions, it was possible to predict the specific gravity at 60° F. of pure 1,3-butadiene. Thus, if it were assumed that the three probable impurities were present in substantially equal proportions, the computed value of pure 1,3-butadiene would be higher than the determined specific gravity of the test material by 0.00007. On adding the buoyancy correction of 0.00003 and subtracting the correction of 0.00005 for the amount of inhibitor present, the final value rounded off to 0.6274 was obtained for the specific gravity (60°/60° F.) of pure 1,3-butadiene. This was considered to be the most nearly correct value for pure 1,3-butadiene at 60° F.

ACKNOWLEDGMENT

The writers wish to acknowledge the helpful suggestions made by various members of the Phillips Petroleum Company Research Department in the development of the experimental method and, in particular, the assistance rendered by L. R. Fruit on all experimental measurements. Acknowledgment is also made both to Phillips Petroleum Company and to the B. F. Goodrich Company for the use of certain analytical techniques.

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Physical Methods of Analysis of Synthetic and Natural Rubber

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In a compounded rubber stock, the ratio of natural to synthetic rubber can be estimated approximately from a knowledge of the phosphorus content of the rubber hydrocarbon. A considerably more exact analysis can be carried out by means of infrared spectroscopic methods, which permit a determination of the type as well as the amount of rubber present. Complete details and comparative results of these two methods of analysis are given, as well as a simple procedure for separating the rubber hydrocarbon of a rubber stock from the carbon black and other compounding ingredients.

REPORTS have been made that more than 3000 kinds of rubberlike synthetics have already been prepared. Obviously, not all are of practical importance, nor can they legitimately be referred to as "synthetic rubbers", but a certain select group of these, as well as still other new synthetics, will survive and prove meritorious. Uses will be found for these new synthetic rubbers both alone and when blended with other synthetics or with natural rubber. The rubber chemists of the future will therefore be faced with the problem of analyzing such mixtures. Because the components of these mixtures are often complex and their degradation products are not well known, it will be difficult, by conventional analytical methods, to establish their identities or the proportions in which they are present.

In anticipation of these difficulties, it was deemed advisable to study the applicability of various physical tools to this and other problems of the rubber industry. In a previous publication (*1*) the authors called attention to the fact that infrared spectroscopy could be of value in differentiating between rubbers and in analyzing rubber mixtures, and pointed out that the infrared absorption spectra of the various rubbers are unique and offer one means of attacking the analytical problem outlined. However, in this preliminary paper the analytical possibilities were merely noted without any attempt to reduce them to practice.

Shortly after the completion of this preliminary study, the authors were called upon by the War Production Board to analyze a series of captured German tires and inner tubes for the Army Ordnance Department, and to determine, if possible, the amounts and types of synthetic rubbers which had been blended with natural rubber in the manufacture of these tires. As a result of this investigation, two satisfactory methods of analysis were developed.

The first, the determination of the phosphorus content, furnishes a simple method for measuring the relative amounts of natural and synthetic rubbers present in an unknown; the second, the application of infrared spectroscopy, permits determination of both the type of each rubber and the amount present. In conjunction with the latter analysis, a method was devised for separating from compounded rubber products a sample of rubber hydrocarbon free of pigment and filler. The details of the preparation and analysis of samples are given, together with a typical set of data obtained in connection with the tire and tube studies.

Although these methods may not necessarily be successful in solving every type of rubber mixture analysis encountered, their value in this particular problem warrants careful consideration in connection with similar problems which may arise in the future.

DETERMINATION OF RATIO OF NATURAL TO SYNTHETIC RUBBER BY PHOSPHORUS CONTENT

The metabolic processes of plants bring about, within the various parts of the plant, the deposition of a great many of the metals commonly found in the soil. In contrast with this, the metals found in any synthetic product are limited to those purposely added and those accidentally introduced by contact with the pieces of processing equipment.

In order to determine whether this difference might prove to be valuable for analytical purposes, samples of natural and synthetic rubbers were subjected to an ultraviolet spectrochemical analysis, using a large Hilger E-1 spectrograph. Table I shows the results of such a comparative emission analysis. It may be seen at once that, whereas the synthetics contain little or no phosphorus, this element is present in the natural rubbers in readily detectable quantities. This clue was followed up in great detail and the exact values for the phosphorus contents were determined through the use of another spectrochemical

Table I. Ultraviolet Spectrochemical Analysis of the Metal Content of Rubber Hydrocarbons

	Natural Rubber					Synthetic Rubber		
	Smoked sheet	Crepe rubber	Tread stock	Carcass stock	Reclaimed tires	Domestic Buna S	German Buna S	Buna S tread stock
	Per Cent Ash							
	(0.27)	(0.17)	(3.6)	(32.5)	(23.2)	(1.49)	(2.11)	(6.3)
Aluminum	2−	2−	2+	2−	3−	2−	2+	2
Antimony	0	1	0	0	2	0	0	0
Barium	1+	1+	1+	1+	3−	2−	2−	1+
Boron	1+	1+	2−	2−	1	1+	1+	2−
Cadmium	0	0	1	1+	2−	0	1−	1
Calcium	2	2	2+	2−	3−	2+	2+	3−
Chromium	1	1	1+	0	2−	1+	1+	2−
Copper	2−	2−	1+	1+	2−	2−	2−	1+
Iron	2−	2−	2	2	2	2	2	2
Lead	1	1	1+	2−	2	1+	1+	1+
Magnesium	2+	2+	3−	2+	3−	2+	3−	3−
Manganese	1	1	1+	1	1+	2−	1+	1
Molybdenum	0	0	0	0	2−	0	0	0
Nickel	1	1	1+	0	1+	1+	1+	2−
Phosphorus	2+	2	2+	2	2	0	1+	0
Potassium	2	2	0	0	2−	2−	0	1+
Silicon	2	2	2+	2	2+	2	2+	3−
Sodium	2	2	2	2	3−	2+	2	2+
Strontium	1+	1+	1	0	2	1+	1+	1
Tin	1	1	1	1	1	1+	1	1
Titanium	1+	1	1+	1	2	1	2	1
Vanadium	0	0	0	0	0	0	2−	0
Zinc	2−	2−	3−	3	2+	2	2−	3−

Ranges for qualitative estimates:

3 = 100 to 1.0%
3− = 10 to 0.10%
2+ = 1.0 to 0.01%
2 = 0.1 to 0.001%
2− = 100 to 1.0 p.p.m.
1+ = 10 to 0.10 p.p.m.
1 = 1.0 to 0.01 p.p.m.
1− = less than 0.1 p.p.m.
0 = metal not detected

tool, the visible light spectrophotometer. The details of the exact procedure followed are given below.

Table II gives the phosphorus contents of a variety of natural and synthetic rubbers. These values show a considerable variation for natural rubbers of different origins, but all may be characterized by high phosphorus (average 400 p.p.m.) when compared with typical synthetics (average 20 p.p.m.). Thus, an exact phosphorus determination should make possible a determination of the ratio of natural to synthetic rubber in an unknown sample.

Table II. Phosphorus Content of Various Rubbers

	P.p.m.		P.p.m.
Natural rubber		Synthetic rubber	
Smoked sheet A	690	Buna S (American)	15
Smoked sheet B	380	Buna A (German)	30
Smoked sheet C	320	Cotton tire cord	210
Smoked sheet D	500	Viscose tire cord	10
Smoked sheet E	400		
Crepe	490		
Pale crepe	350		
Reclaimed tube	390		
Guayule	200		

Figure 1 was plotted in order to show that the phosphorus could be determined accurately enough to allow the content of this element to be used as a yardstick and as a direct measure of the amount of natural rubber present. (If the origin of the natural rubber is unknown, the slope of this curve is not determinable, and the method is only roughly quantitative. On the other hand, if a sample of the natural rubber used in compounding is available for a phosphorus test, a much higher degree of accuracy may be expected.) In spite of the variation of the phosphorus content of natural rubbers of different origin, a reasonable first guess at a mixture of unknown constitution may be based upon the following generalization:

High phosphorus (300 to 450 p.p.m.)	= natural
Medium phosphorus (100 to 250 p.p.m.)	= natural + synthetic
Low phosphorus (0 to 50 p.p.m.)	= all synthetic

As will be seen from Table IV, the above method provides a very simple approximate analysis. The agreement between this and the more exact infrared method is indeed surprising. However, the method is always open to the uncertainty that phosphorus may have been introduced in processing and not been eliminated in the sampling procedure.

Although any sensitive method for determining the phosphorus will be satisfactory, the spectrochemical procedure detailed below was found to be most suitable. This colorimetric method is based upon many literature references of which Zinzadze (*10*) and Goodloe (*6*) are typical. An intense blue color is produced in solutions containing phosphorus by reduction of phosphomolybdate, the intensity of the color being directly proportional to the phosphorus content of the solutions. In this laboratory, the color values or per cent transmissions were measured on a General Electric recording spectrophotometer. The blue color which develops has a rather broad spectral width which can be measured satisfactorily by means of any ordinary comparison colorimeter, although the increased precision of the more accurate photoelectric instruments is to be preferred. Figure 2 shows a series of transmission curves for standard phosphorus solutions.

Preparation of Sample. All glassware and reagents must be free of phosphorus and a suitable blank or control must be carried through the complete procedure.

Other possible sources of extraneous phosphorus are the cord and plasticizer used in fabrication. [It is interesting in this connection to note (Table II) that cotton, or natural, fibers are high in phosphorus, whereas synthetic fibers, rayons, etc., have a low content, thus enabling a distinction to be made between these two types of fibers. Undoubtedly other cases of natural versus synthetic materials may arise in which this general method might be of help.] Therefore, it is advisable that all cords, if present, be removed by the following procedure: A section of the rubber (tire) is shredded in a two-roll mill, then mixed with water in a Waring Blendor. After 5 to 10 minutes' stirring, the cord can be partially separated by decantation. The remaining cord is digested at about 4° C. for 18 hours with an excess of cuprammonium solution [see Clibbens and Gaeke (*5*) and the committee of the American Chemical Society (*4*) for preparation of this solution]. The material is washed free of cuprammonium with water and then dried. If the sample is a prepared rubber stock, all phosphate-containing plasticizers must be removed by refluxing the shredded rubber for 8 hours in a Soxhlet extractor with a solvent composed of 68% chloroform and 32% acetone.

For maximum photometric accuracy, a weight of rubber should be taken such that a phosphorus content up to 0.080 mg. is contained. With synthetic rubbers, a 1-gram sample is suitable, whereas, with natural rubber, approximately 0.1 gram is used. In order to ensure a representative sample, a larger weight is taken and subjected to the preliminary preparation. The color value of a 1-gram equivalent is then measured to furnish a rough idea of the phosphorus content. With this as a basis, a weight of sample is chosen according to the above criterion and is measured accurately.

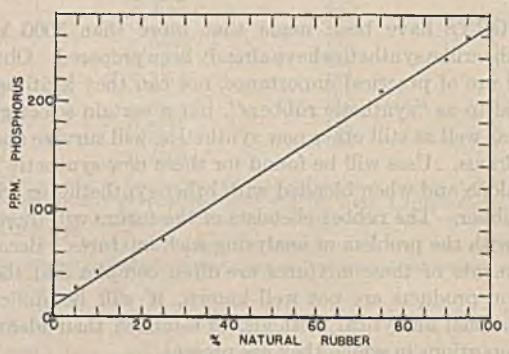

Figure 1. Phosphorus in Carcass Stock Compounded with Natural Rubber and Buna S

Formula of Stock	
Rubber hydrocarbon	100
Zinc oxide	50
Stearic acid	1
Pine tar	1.5
Agerite resin	1.5
Sulfur	2.0
Altax	1.25

Preferably, the phosphorus values should be calculated to the weight of rubber hydrocarbon, the carbon, zinc oxide fillers, etc., having been removed previously. This was not always done in the authors' work, as the carbon analysis was done elsewhere; hence, the phosphorus content of the samples reported here was based on the entire rubber sample.

Ashing of Sample. The weighed sample of extracted rubber is ashed in a suitable crucible in a controlled furnace at a temperature not higher than 600° C. until free of organic material. This must be done slowly enough to prevent the material from kindling. It was not found necessary to add a phosphorus fixative in the case of rubber materials, as no increased phosphorus values were obtained by adding zinc oxide to prevent the volatilization of phosphorus. After cooling, the residue is dissolved by boiling in dilute sulfuric acid in a quantity just sufficient to dissolve the soluble materials. Excess acid is to be avoided, since the final acidity is very important, but an excess of acid may be added and later neutralized. A slight turbidity which may be removed by filtering remains in some cases as a result of the presence of a siliceous material. The clear solution of the ashes is transferred to a 50-ml. Pyrex volumetric flask and the volume is adjusted to about 40 ml.

Development of Color. Although many procedures are described in the literature, the following modification was used here.

Standard Solutions. Solution I, ammonium molybdate, 5.45 grams [$(NH_4)_6Mo_7O_{24}.4H_2O$], dissolved by warming with water and made to 100 ml.

Solution II, 10*N* sulfuric acid (282 ml. of concentrated sulfuric acid diluted to 1000 ml.), checked by titration.

Solution III, stannous chloride. A stock solution of stannous chloride dehydrate is 40 grams dissolved in concentrated hydrochloric acid (density 1.18) and made up to 100 ml. (stable for months). For use, it is diluted 200 times with distilled water (stable for one day).

Analytical Procedure. To the solution prepared according to instructions above, 2.5 ml. of Solution I and 5 ml. of Solution II are added and mixed thoroughly, and 2 ml. of Solution III are added with constant swirling. The solution is made to volume (50 ml.) and mixed thoroughly. (The final acidity of the 50-ml. sample should be 1 *N* sulfuric acid. If excess acid is used in dissolving the ash, it may be neutralized at that point or the amount of Solution II may be reduced to give the final acidity stipulated.) A blank is carried through the same procedure to check for the presence of phosphorus in the reagents.

Determination of Phosphorus Content. The per cent transmission of the solution in a 1-cm. thick cell at 700 mμ is measured exactly 20 minutes after adding Solution III. The amount of phosphorus in an unknown may be determined from a calibration curve which shows the per cent transmission plotted as ordinates versus the phosphorus content of known standard solutions as abscissas. The calibration curve may be prepared from a series of transmission curves such as those of Figure 2.

Notes on Phosphorus Analysis. When a molybdate is added to a solution containing orthophosphate according to the method described above, an insoluble phosphomolybdate is formed. However, because of the high dilution, a precipitate is not apparent. The addition of a reducing agent (stannous chloride) causes a reduction of the phosphomolybdate and gives an intense blue color. Under the proper conditions, the molybdenum reagent necessary as an excess is not reduced.

The factors which affect the color are:

1. The acid concentration is very important. In the presence of too much acid, a light color is produced. Conversely, if too little acid is used, the molybdate reagent itself will be reduced, causing dark colors. In the method used, a 20% decrease in acidity results in a 10% increase in color intensity, while a 10% increase in acidity results in a 10% decrease in color intensity. Soluble silica also produces a blue color with the above reagent if the silica concentration is high or the acidity is insufficient to suppress the ionization of the silicic acid. The acidity chosen as optimum in this investigation (1*N* sulfuric acid) is such that silica up to 2000 p.p.m. does not interfere.
2. The molybdate concentration is the next most critical factor. An increased molybdate concentration results in a higher sensitivity to phosphorus, but also an increased blank. A high blank reading is not desirable when low concentrations of phosphorus are present. A 50% increase in the molybdate concentration, as used above, results in approximately a 15% increase in the color intensity. A 20% decrease in molybdate concentration results in approximately a 10% decrease in color intensity.
3. The stannous chloride concentration is not very critical. A ±50% change in the amount of stannous chloride influences the phosphorus result not more than ±5%.
4. Under the given conditions, the color increases for 5 to 10 minutes after the stannous chloride is added and bleaches slowly thereafter. It is recommended therefore that the color be measured 20 minutes after addition of the stannous chloride.
5. The following sources of interference have been considered, and methods of reduction or elimination are recommended where necessary:

Ferric ion up to 6 p.p.m. does no harm. Fifteen parts per million slightly inhibit color development, while larger amounts of ferric ion cause very rapid fading of the color. Ferrous ion causes no harm. A Jones reductor with metallic cadmium gives best results for prevention of interference from ferric ion (*7*).

The presence of more than 20 p.p.m. of titanium causes interference by retarding the rate of color development.

Arsenates give the same color as phosphates and the intensities are inversely proportional to the molecular weights. Reduction with sodium bisulfite eliminates the influence of arsenates (20 mg. of arsenic pentoxide per 50 ml. may be taken care of by reduction to arsenic trioxide).

Nitrates up to 100 p.p.m. have no effect; 200 p.p.m. reduce the color about 10%.

Sulfates in large amounts interfere, presumably by depressing the ionization of the sulfuric acid.

Tartaric and citric acids interfere above 20 p.p.m., with inhibition of maximum color. They may be removed by oxidation with permanganate.

Aluminum and manganese in reasonable amounts do not interfere.

Calcium and magnesium up to 1000 p.p.m. have no effect.

Nickel up to 1000 times the phosphorus content does not interfere except for its own color.

Trichloroacetic acid begins to interfere with the maximum color development at concentrations above 4% in the final mixture. Acetic acid shows practically no effect.

Hydrochloric acid has a tendency to lessen color stability and retards or inhibits maximum coloration.

DETERMINATION OF TYPES AND AMOUNTS OF RUBBERS BY INFRARED SPECTROSCOPY

Recent publications (*1*, *2*, *3*, *8*) have described in great detail the methods and applications of infrared spectroscopy to the identification and analysis of many types of organic materials. It is sufficient here to point out the two salient characteristics of infrared absorption in order that the basis for analysis can be understood.

In the first place, the infrared absorption spectrum (a plot of per cent transmission as ordinate versus frequency in $cm.^{-1}$) of a material is a unique characteristic of the material and cannot be duplicated by another compound. Some of the absorption bands can be ascribed to particular atomic groups within the molecule while others, characteristic of the molecule as a whole, are particularly useful for such studies as differentiating isomers. Thus it is to be expected that the phenyl group in Buna S would give rise to absorption bands which would not be present in natural rubber, while conversely, the methyl groups of natural rubber would cause a characteristic absorption which would not be observed in Buna S. Moreover, it is to be expected that there will be further bands characteristic of the molecule as a whole which will assist in the differentiation.

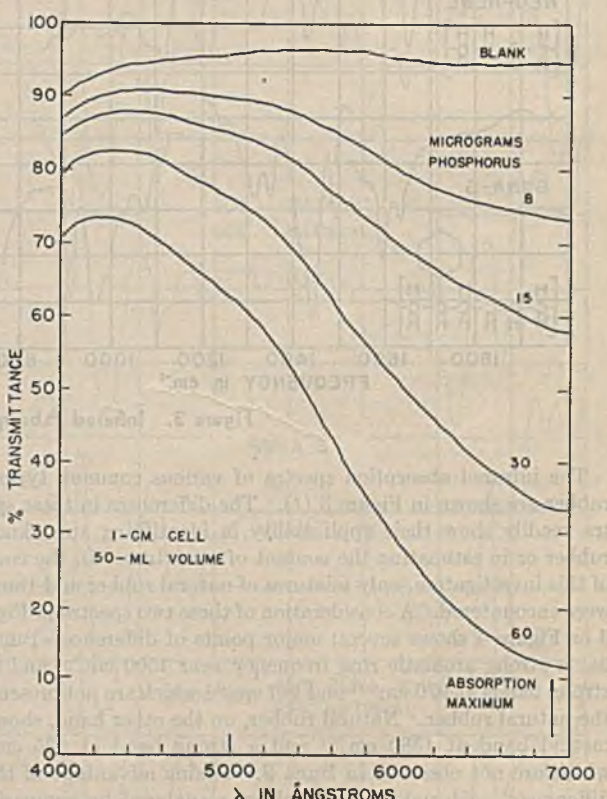

Figure 2. Spectrophotometric Determination of Phosphorus

Calibration of phosphorus as reduced phosphomolybdate
Final solution 1*N* in sulfuric acid, MoO_3 0.13 gram per 50 ml., $SnCl_2.2H_2O$ 0.006 gram per 50 ml.

T %	−log *T*	Blank	Net −log *T*	Phosphorus *Micrograms*	P, −log *T* *Microgram*
94.5	0.024	..	..	None	..
73	0.137	0.024	0.113	8	0.0141
58	0.237	0.024	0.213	15	0.0142
34	0.469	0.024	0.445	30	0.0148
12.5	0.903	0.024	0.879	60	0.0146

In the second place, so long as no intermolecular action occurs, the spectrum of a mixture of rubbers will be simply the spectra of the pure components combined in the proportion in which the materials themselves are present. Hence, it is to be expected that an unknown mixture can be analyzed by direct measurement of the strength of absorption bands unique to each component, or by comparison of the absorption spectra of the unknown with those of a series of known prepared standards.

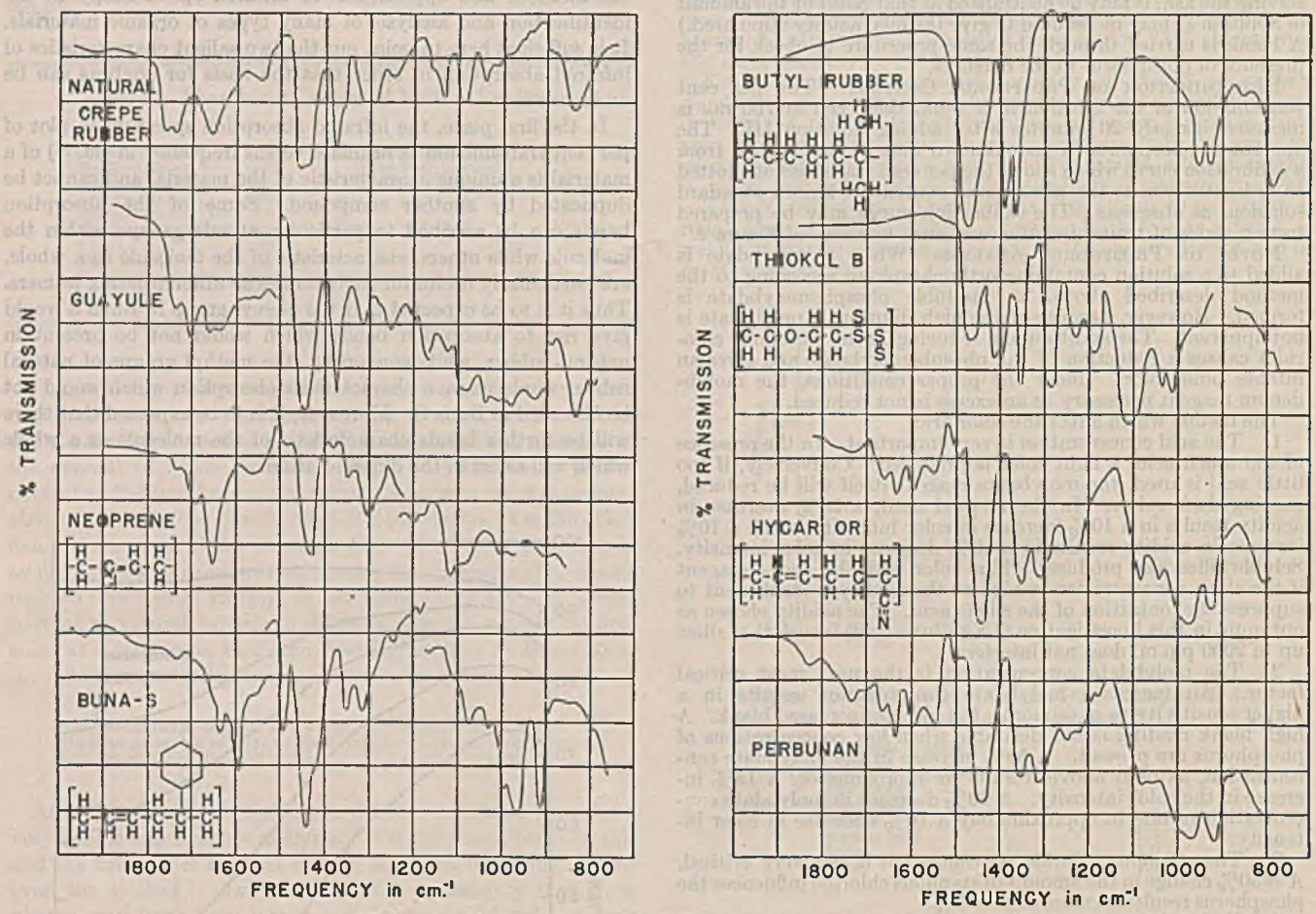

Figure 3. Infrared Absorption Spectra of Rubber Hydrocarbons

The infrared absorption spectra of various common types of rubber are shown in Figure 3 (*1*). The differences in these spectra readily show their applicability in identifying an unknown rubber or in estimating the content of a mixture. In the course of this investigation, only mixtures of natural rubber and Buna S were encountered. A consideration of these two spectra in Figure 3 or Figure 4 shows several major points of difference—Buna S has a strong aromatic ring frequency near 1500 cm.$^{-1}$ and two strong bands at 970 cm.$^{-1}$ and 917 cm.$^{-1}$ which are not present in the natural rubber. Natural rubber, on the other hand, shows a methyl band at 1380 cm.$^{-1}$ and a strong band at 835 cm.$^{-1}$ which are not observed in Buna S. Taking advantage of these differences, each unknown sample was analyzed by comparison of its spectrum with the spectra of a series of known mixtures compounded and treated in approximately the same way as the unknown materials. The method is outlined roughly in Figure 4, showing the absorption spectra of natural rubber, 50% natural and 50% Buna S, pure Buna S, and an unknown tire tread. It can be seen from the 50–50 mixture that the unique bands mentioned above are all present with a strength proportional to the component concentration.

In the actual analysis, a great many intermediate standard spectra were obtained. An unknown was matched with one of this series by a comparison of the absorption intensities $\frac{1500\ \text{cm.}^{-1}}{1380\ \text{cm.}^{-1}}$ and $\frac{917\ \text{cm.}^{-1}}{835\ \text{cm.}^{-1}}$. By this method, an unknown such as that shown in Figure 4 could be estimated to be 85% natural rubber, 15% Buna S. All absorption spectra were taken with samples smeared on salt plates and a high resolution spectrometer (*2*) was employed. The accuracy of the method is limited to 5 to 10% because of the inability to make smear samples of constant thickness. Greater accuracy, if desired, could be obtained by making per cent transmission measurements at the chosen frequencies according to some of the more involved analytical methods referred to above (*2*, *3*, *8*). In order to justify the careful treatment, it would be necessary to study the unknowns and standards as solutions of known concentration in a suitable solvent which would transmit infrared radiation at the analytical frequencies chosen. This more careful treatment would still require a preliminary complete absorption spectrum of each unknown, in order to furnish a qualitative analysis for the various rubber materials which are present.

Sample Preparation. The methods described above are standard procedures and are given in detail in the references. Considerable time, however, had to be devoted to finding a means of converting a piece of tire stock to a sample whose infrared absorption spectrum could be measured. All attempts to obtain the spectra of the tire stocks directly, either by microtoming a thin section or by a smear from a solution of the stock material, were unsuccessful. In order to obtain a satisfactory spectrum, it is necessary to extract the rubber hydrocarbon free of plasticizer, filler, etc. The method finally devised was completely satisfactory for the authors' purpose and should be of great value in

any situation where it is desirable to separate filler and rubber without destruction of the rubber hydrocarbon. Therefore, this separation by means of *p*-cymene and xylene solution is completely described below.

After this separation, the rubber is present as a solution in the hydrocarbon solvents. These solvents are removed by vacuum distillation until the rubber is a gummy solid that will not flow at room temperature. This gum is then washed four or five times with hot acetone to remove the last traces of plasticizer and solvent.

In most cases the state of the rubber is such that it can easily be smeared on a rock salt plate. The desired thickness is obtained by scraping the film with a razor blade until the 1450 cm.$^{-1}$ CH band shows a transmission of about 10%. If the rubber does not spread easily, it may be softened with a volatile solvent such as carbon tetrachloride. The film is then dried in a vacuum oven to remove the last traces of solvent. Finally, the absorption spectrum of the sample is obtained throughout the spectral region of interest.

DISSOLVING RUBBER HYDROCARBON AND SEPARATING IT FROM COMPOUNDING INGREDIENTS

In the case of vulcanized natural rubber compositions, various solvent methods of determining the amount of rubber hydrocarbon have been used with some success. These methods, however, are somewhat involved and time-consuming. Even the A.S.T.M. method requires that the solution stand overnight to allow the mineral fillers and pigments to settle before filtration. If the rubber composition contains highly dispersed, exceedingly fine pigments, such as carbon black, separation of the pigments by filtration or centrifuging is usually impossible.

Since many present-day rubber products, particularly tire tread compositions or compounds, contain large amounts of carbon black, none of the above-mentioned methods appeared promising for separation of the rubber from the carbon black in suitable condition for infrared analysis. Accordingly, research on this question was initiated.

Fearing that destruction or decomposition of the rubber hydrocarbons might result from the use of the conventional high-boiling solvents used in the above separation methods, a number of low-boiling solvents were tried: ethylene dichloride, toluene plus piperidine, *o*-nitroanisole, Dispersing Oil No. 10 (Barrett) plus xylene, and Circolight Process Oil (Sun Oil Co.) plus xylene. These experiments were unsuccessful.

Method I. In the next attempts, which were somewhat more successful, xylene plus a small amount of thio-β-naphthol boiling under reflux was found to dissolve natural or synthetic tire tread stocks in 5 to 6 hours. To accomplish this, 1 gram of chloroform-acetone–extracted tread stock was heated in 100 cc. of xylene plus 0.3 gram of thio-β-naphthol at 140° C., until solution was complete.

However, this solution did not permit easy separation of the carbon black unless a combination of centrifuging and slight alcohol precipitation was used. (By adding alcohol to the solution, a small amount of rubber is precipitated in order to drag down the carbon black during centrifuging. There is some danger that this precipitation may be selective and thus change the composition of the residual rubber.) A sufficient separation of the carbon black can be made in this manner to permit fairly satisfactory infrared analysis. In the case of carcass stocks containing only zinc oxide as a filler, the pigment was easily separated by settling or centrifuging. This method was not completely satisfactory, however, because of carbon black troubles and the difficulty of removing the peptizer without loss of the rubber itself.

The search for a better means of carbon black separation was therefore continued. The cresol method (*9*) was tried with some success, although there was considerable oxidation of the rubber at the high reflux and distillation temperature required. The use of *p*-cymene in place of the cresol with digestion at about 160° to 170° C. gave a satisfactory solution in about 4 hours and caused no noticeable oxidation of the rubber. On dilution with the benzene and 70° Bé. rubber solvent gasoline, the supernatant liquid showed clearing in a few minutes and could be filtered free of the carbon black after standing 10 minutes. This method was further altered slightly by using 250 cc. of *n*-hexane instead of 300 cc. of 70° Bé. gasoline for diluting the solution. Again the carbon black separated easily. In order to determine whether or not the same dilution technique would work with xylene as the solvent, a solution of the same tread stock was made with 30 cc. of xylene plus a small amount of thio-β-naphthol, but on diluting with benzene and hexane, the carbon black would not separate from the solution.

Further experiments showed that the combined use of *p*-cymene and xylene gave the most successful results and led to a satisfactory method.

Method II (adopted for solution of rubber and separation of pigments). Sheet the sample to a thickness of approximately 0.375 cm. (0.15 inch) on a tight cold 15 × 30 cm. (6 × 12 inch) laboratory mill.

Extract the plasticizer, etc., from 4 grams of the sheeted sample with a mixture of 32% by volume of acetone and 68% by volume of chloroform for a minimum of 7 hours, or until the extracting liquid no longer shows color. This size of sample is sufficient to permit repeat analyses.

Place 1.0 gram of the dried, extracted sample in a 400-cc. rubber extraction flask with 25 cc. of *p*-cymene and 5 cc. of xylene. Heat on a steam bath at about 70° to 80° C. for 1 hour, then on a

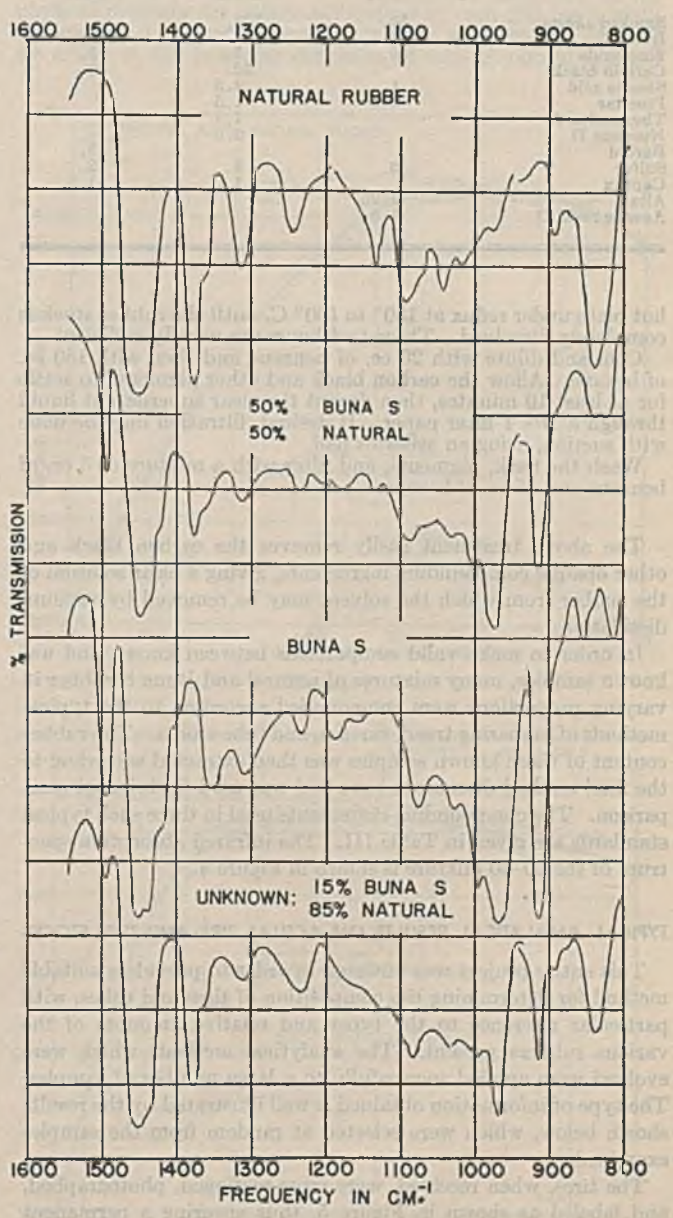

Figure 4. Outline of Infrared Analytical Method

Spectra of prepared standards and unknown sample

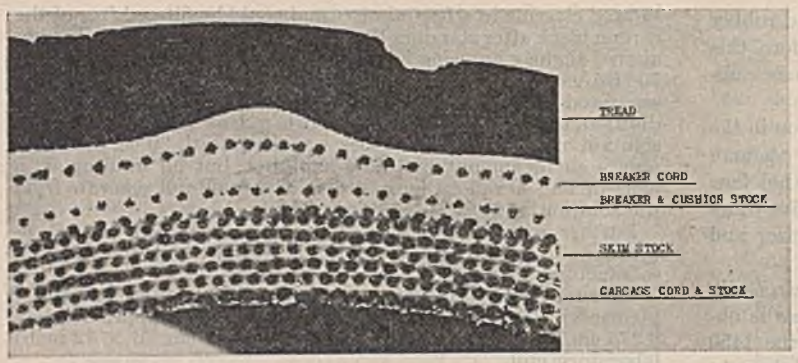

Figure 5. First Step in Sampling Procedure

Labeled cross section of tire to be analyzed for rubber content

Table III. Composition of Typical Rubber Stocks

	Stocks		
	87-1	A-912	A1-141
Smoked sheets	50.	100.	
Buna S	50.		100.
Zinc oxide	50.	5.	5.
Carbon black		50.	50.
Stearic acid	1.	2.5	
Pine tar	1.5	1.5	
Thermoflex A		1.4	
Neozone D		0.6	
Bardol			5.
Sulfur	2.	3.	2.
Captax		1.2	1.5
Altax	1.25		
Agerite resin D	1.5		

hot plate under reflux at 150° to 160° C. until the rubber stock is completely dissolved. Three to 4 hours are usually sufficient.

Cool and dilute with 20 cc. of benzene and then with 150 cc. of hexane. Allow the carbon black and other pigments to settle for at least 10 minutes, then decant the clear supernatant liquid through a No. 1 filter paper. If desired, filtration may be done with suction, using an asbestos pad.

Wash the flask, pigments, and filter with a mixture of 5 cc. of benzene and 45 cc. of hexane.

The above treatment easily removes the carbon black and other opaque compounding ingredients, giving a clear solution of the rubber from which the solvent may be removed by vacuum distillation.

In order to make valid comparisons between known and unknown samples, many mixtures of natural and Buna S rubber in varying proportions were compounded according to the typical methods of preparing tread, carcass, and tube stocks. The rubber content of these known samples was then extracted according to the final method discussed above and was used for infrared comparison. The compounding ingredients used in three such typical standards are given in Table III. The infrared absorption spectrum of the 50–50 mixture is shown in Figure 4.

TYPICAL ANALYTICAL RESULTS ON ACTUAL TIRE AND TUBE STOCKS

This entire project was initiated in order to provide a suitable method for determining the composition of tires and tubes, with particular reference to the types and relative amounts of the various rubbers present. The analytical methods which were evolved were applied successfully to a large number of samples. The type of information obtained is well illustrated by the results shown below, which were selected at random from the samples examined.

The tires, when received, were cross-sectioned, photographed, and labeled as shown in Figure 5, thus ensuring a permanent record of the tires and a ready means of identifying the various samples chosen. Wherever possible, samples of the tread, cushion, breaker, and carcass stocks were removed from each tire, extracted, prepared for study, and then subjected to the two analytical procedures.

In Table IV, the results obtained on a few typical German tires and inner tubes are presented. In view of the spread in the phosphorus contents of natural rubbers from various sources (Table II), a mean value of 400 p.p.m. was used in making the analytical calculations from phosphorus determinations. The agreement between the infrared and the phosphorus methods is therefore all the more gratifying.

ACKNOWLEDGMENT

In conclusion, the authors would like to express their appreciation to the several other members of the staff of this laboratory who helped materially in the establishment of these analytical methods.

Table IV. Analysis of Captured German Tires

Tire No.	Phosphorus		Infrared	
	Natural		Natural	Buna S
	P.p.m.	%	%	%
Tread Stocks				
FT 61	280	75	100	0
99	30	0	0	100
100	20	0	0	100
101	10	0	0	100
102	10	0	0	100
103	30	0	0	100
104	25	0	0	100
105	20	0	0	100
106	27	0	0	100
107	3	0	0	100
Carcass Stocks				
FT 61	340	100	100	0
99	340	100	100	0
100	380	100	100	0
101	430	100	100	0
102	130	25	20	80
103	190	50	50	50
104	230	60	50	50
105	200	50	80	20
106	305	80	100	0
107	190	50	50	50
Cushion and Tubes				
FT 61[a]	290	75	100	0
99[a]	270	70	100	0
101[a]	450	100	...	...
102[b]	200	50	75	25
103[b]	255	65	75	25
104[b]	285	70	85	15
105[b]	295	75	100	0
106[b]	260	70	100	0
107[b]	290	75	100	0

[a] Cushion.
[b] Tubes.

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Determination of Rate of Cure for Natural and Synthetic Rubber

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The tensile strength at a given marked undercure divided by the maximum tensile appears to be a convenient index of rate of cure. This index is called the tensile ratio. In comparing two stocks, the one having the higher tensile ratio is the faster curing. The proper undercure to use in calculating the tensile ratio has been found to be in the range of roughly 60% of the maximum tensile, which for many stocks occurs at about one fourth the time to reach maximum tensile. The tensile ratio is very easy to determine, requires no special equipment, and can be determined fairly accurately. In addition, it agrees well with generally accepted indexes of cure rate for typical natural rubber and GR-S formulations.

THE methods that have been proposed for determining the rate of cure of natural rubber stocks include:

Time to reach some maximum physical property, such as tensile strength, tensile product (*12*, *16*), modulus at a definite strain, aged tensile, or some property of practical importance for a given compound.

Tests depending on temperature susceptibility like T-50 (*5*, *6*, *7*, *11*, *14*) or zero degree set (*3*).

Chemical tests, such as determination of the amount of combined sulfur (*12*).

Special tests based on physical measurements, such as time to reach the break in the modulus at a given strain *vs.* cure-time curve (*4*), time to reach the cure giving "reasonable snap with substantially unimpaired tear" (optimum hand tear method) (*2*), and time to reach minimum reduced residual elongation (*8*).

Of these methods, the T-50 test and the time to reach maximum tensile have been most widely accepted and have proved very useful, but leave something to be desired. The T-50 test requires special equipment not always available in smaller rubber laboratories, while the time to reach maximum tensile, although obtainable from tensile data which in most cases would have to be determined anyhow in order to evaluate the compound, is difficult to determine accurately, as the tensile *vs.* cure-time curve usually has a flat maximum.

In GR-S the need for a convenient index of rate of cure is more urgent. The T-50 test is not applicable to GR-S compounds (*9*) and as a rule the tensile *vs.* cure-time curve has an even flatter maximum than in rubber, so that the accuracy of determining the time to reach maximum tensile is even lower.

In considering the question of obtaining a satisfactory index of rate of cure, it will be helpful to define the terms "optimum cure", and "state of cure", "rate of cure".

Optimum cure for a given physical property may be defined as the time required to reach the maximum or optimum value for that property—for example, the optimum cure with respect to rebound would be the cure time at a specified cure temperature to bring the stock to its maximum rebound. The property most generally used is tensile strength and the optimum cure for tensile strength is often referred to simply as the optimum cure.

State of cure has been defined as the position of the cure in question in a series of cures (*15*). It seems more precise to define state of cure with respect to a given physical property as that fraction of the maximum value of the property shown by the cure in question (*16*). For example, if the 30-minute cure of a certain tread stock has a rebound of 47% and if the maximum rebound for this tread stock is 50%, then the state of cure of the 30-minute cure with respect to rebound can be expressed as 47/50 = 0.94. It may be useful in some instances to distinguish undercures from overcures; we may, therefore, indicate overcures by placing a negative sign before the state of cure.

Rate of cure may be defined as the time required to reach a given state of cure compared to some standard or control stock. Specifically, rate of cure referred to a given state of cure equals time to reach that state for the control divided by the time for the sample to reach the same state. In the previous example, if the standard stock reaches a state of cure of 0.94 based on rebound in 20 minutes, the rate of cure of the sample tread stock would be 20/30 = 0.67, since the sample stock required 30 minutes to reach the 0.94 state.

Optimum cure and state of cure depend on the physical property used to determine curing characteristics. Rate of cure may depend in addition on the state of cure at which the rate is measured. From a practical viewpoint the basic physical property is the useful life of a satisfactory commercial product made from the compound in question. If we then take useful life as the physical property for determining state of cure and use a state of cure of 1.0 (optimum cure) at which to measure rate of cure, we arrive at the following definition of rate of cure at a given

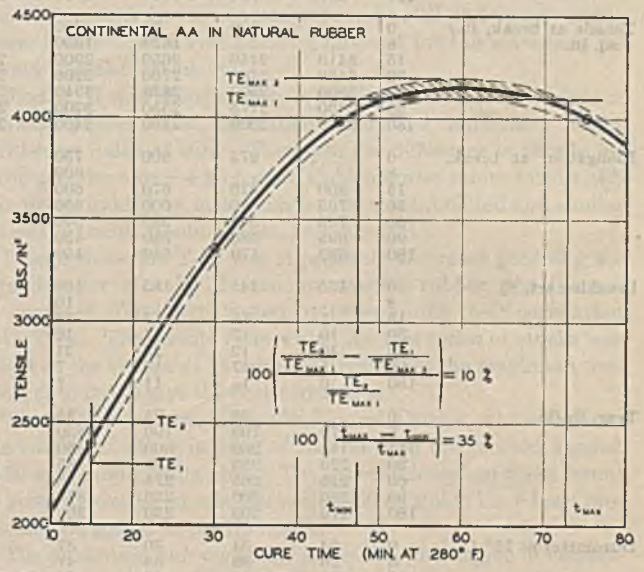

Figure 1

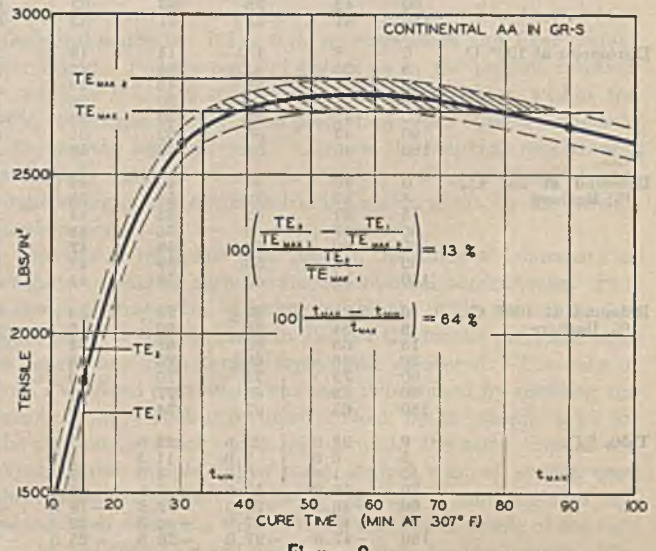

Figure 2

cure temperature—rate of cure for any stock is the cure time required to reach maximum useful life for a standard control stock divided by the cure time required to reach maximum useful life for the sample stock.

While the foregoing definition is precise, it is not very useful in actual laboratory work. Some index of rate of cure is therefore desired which correlates closely with rate of cure as defined above but can be determined conveniently and accurately. In the absence of data connecting any proposed index of rate of cure with actual useful life tests it may be considered satisfactory to show that the proposed index correlates closely with indexes of rate of cure, the usefulness of which has already been established.

Table I. Natural Rubber

Formula		Gum	Pptd. Whiting	MT	Continex	Continental AA	Continental D	Continental R-40
Smoked sheet		100	100	100	100	100	100	100
Zinc oxide		7.85	7.85	7.85	7.85	7.85	7.85	7.85
Pine tar		3	3	3	3	3	3	3
Stearic acid		3.3	3.3	3.3	3.3	3.3	3.3	3.3
Sulfur		2.81	2.81	2.81	2.81	2.81	2.81	2.81
Mercaptobenzothiazole		0.743	0.743	0.743	0.743	0.743	0.743	0.743
Precipitated whiting		.....	78	.....	.....	.....	.....	.....
Medium thermal (MT)		.....	.....	50	.....	.....	.....	.....
Continex (SRF)		.....	.....	.....	50	.....	.....	.....
Continental AA (EPC)		.....	.....	.....	.....	50	.....	.....
Continental D (MPC)		.....	.....	.....	.....	.....	50	.....
Continental R-40 (CC)		.....	.....	.....	.....	.....	.....	50
				Cure at 280° F.				
	Min.							
Modulus at 400% elongation, lb./sq. in.	0	....	....	....	....	....	....	....
	8	....	340	300	900	350	250	180
	15	180	870	720	1420	1070	720	370
	30	280	960	1060	1920	1620	1060	770
	60	300	1100	1250	2120	2100	1430	1690
	90	390	900	1350	2200	2330	2360	1800
	180	390	780	1320	2300	2675	2630	2470
Tensile at break, lb./sq. in.	0	0	100	70	200	250	370	380
	8	....	....	1620	1950	600	750	460
	15	2410	2450	2650	2900	2400	2040	1200
	30	3580	2400	2700	3200	3340	2660	2600
	60	3800	2300	2820	3340	3750	3290	3300
	90	3490	2175	2550	3200	3900	3880	3360
	180	2670	2000	2450	2450	3750	3780	3170
Elongation at break, %	0	....	975	900	750	860	975	1100
	8	....	....	....	600	775	800	850
	15	800	610	670	600	600	650	800
	30	765	600	600	550	600	640	720
	60	755	580	585	540	600	630	600
	90	695	580	550	450	565	570	600
	180	680	570	550	450	530	535	490
Breaking set, %	0	135	145	185	180	220	205	275
	8	....	....	....	10	120	105	180
	15	7	15	17	12	17	24	....
	30	10	17	17	16	25	25	35
	60	10	12	22	21	30	27	27
	90	5	17	15	7	27	32	30
	180	0	15	11	7	21	25	17
Tear, lb./in.	0	....	25	25	35	40	45	50
	8	170	100	100	150	65	90	65
	15	180	260	300	480	440	135	125
	30	220	250	325	485	800	250	200
	60	225	285	275	485	865	600	680
	90	180	200	230	370	770	730	680
	180	170	200	230	300	635	730	540
Durometer at 25° C.	0	24	34	30	37	40	39	45
	8	26	39	38	47	45	45	48
	15	34	48	45	54	52	51	51
	30	39	54	50	58	58	60	59
	60	41	56	51	60	60	62	63
	90	42	55	53	62	65	69	66
	180	41	55	51	62	69	70	70
Durometer at 100° C.	0	8	15	14	18	21	20	30
	8	35	44	38	40	28	28	37
	15	36	50	45	49	42	43	41
	30	41	56	50	56	56	54	50
	60	41	57	52	58	59	55	61
	90	42	55	52	61	63	65	61
	180	40	53	49	60	65	67	64
Rebound at 25° C., %, Bashore	0	46	35	46	43	31	30	23
	8	49	39	51	38	31	32	23
	15	51	42	52	42	30	33	....
	30	57	52	55	48	33	34	27
	60	60	55	58	47	37	32	34
	90	58	53	59	47	36	32	32
	180	56	53	58	45	34	32	28
Rebound at 100° C., %, Bashore	0	28	27	28	26	23	23	23
	8	54	58	60	48	33	34	25
	15	63	65	62	53	43	40	....
	30	70	69	73	63	48	48	33
	60	75	71	75	60	52	46	48
	90	68	68	77	61	50	46	48
	180	65	67	74	60	47	47	41
T-50, ° C.	0	23.0	22.5	22.5	21.0	22.0	21.0	19.8
	8	5.0	7.0	11.5	12.5	....	18.5	20.2
	15	−1.0	0.0	4.5	5.5	12.5	15.3	18.0
	30	−18.0	−12.0	−6.5	−6.5	1.5	3.0	9.5
	60	−46.5	−24.0	−22.8	−18.0	−9.3	−7.2	3.3
	90	−47.5	−27.0	−26.5	−22.0	−13.7	−10.0	−0.5
	180	−47.8	−27.0	−26.5	−25.5	−19.0	−15.8	−3.5

TENSILE RATIO

In order to determine which physical properties most closely fulfill the above criteria for a useful index of rate of cure, the indexes suggested by previous investigators and other combinations of physical properties mentioned below were examined in natural rubber and GR-S. The best index was found to be the tensile ratio, which is defined as the tensile strength at a marked undercure divided by the maximum tensile strength. The undercure best suited for this purpose was found to be the cure giving a tensile strength of roughly 60% of the maximum tensile. For many tread type stocks the desired cure is roughly one fourth the time to reach maximum tensile. For example, for a series of stocks of this type which reach maximum tensile in about 60 minutes—say between 30 and 90 minutes—the proper undercure for calculating the tensile ratio would be about a 15-minute cure.

A very definite objection to the use of the time to reach some maximum property such as maximum tensile as an index of rate of cure is the inaccuracy involved in determining this time when the tensile *vs.* cure-time curve has a flat maximum. The tensile ratio is, of course, not subject to this difficulty, as the flatter the maximum in the tensile *vs.* cure-time curve, the easier it is to determine accurately the value of the maximum tensile and hence the tensile ratio.

In Figures 1 and 2 the effect of the form of the tensile maximum on the accuracy of determining the time to reach maximum tensile is illustrated. In both figures the solid line gives the tensile strength as determined from the average of four identical stocks cured simultaneously at the indicated cures. From the deviations of the individual stocks from the average, the probable error (0.67 times the standard deviation) at each cure was calculated and the two broken lines correspond to the average value minus the probable error and the average plus the probable error.

Thus the two broken lines represent the 50% confidence limits for the tensile at various cures. The stock used for this purpose in Figure 1 was Continental AA in the natural rubber formulation given in Table I, while in Figure 2 Continental AA in the GR-S formula given in Table II was used. For the possible tensile *vs.* cure-time curves that can be drawn within the broken lines, the maxima can be anywhere in the shaded areas, so that the time to reach maximum tensile can vary from $t_{min.}$ to $t_{max.}$ The extreme per cent variation is therefore, as shown on the figures, 35% for the rubber stock and 64% for the flatter GR-S curve. From the same figures the extreme per cent variation for the tensile ratio can also be calculated as indicated on the figures and is 10% for rubber and 13% for GR-S. (These values are only approximate and illustrative. Since the experimental errors responsible for the variation in the individual curves are not entirely independent, the confidence limits for the various calculations may be somewhat different and the figures should not be considered as indicating a precise comparison of the accuracy of the tensile ratio and time to reach maximum tensile.)

DETERMINATION OF RATE OF CURE OF NATURAL RUBBER

In order to determine how satisfactory an index of rate of cure was furnished by the tensile ratio, various pigments were milled into a typical natural rubber formula, 26 volumes of pigment per hundred volumes of rubber being used in all cases. In Table I the formulas of the stocks used and the experimental test results are given. The results include tests on uncured stock and on a range of cures from 8 to 180 minutes and permit comparison of the tensile ratio and various indexes of rate of cure which have been used or recommended for natural rubber. The time to reach maximum tensile, tensile product (*12*, *16*) (tensile multiplied by elongation), tear, and rebound were determined from the data. Likewise the time to reach minimum reduced residual elongation (breaking set divided by tensile) (*8*), break in modulus at 400% elongation (*4*), and optimum cure as determined by hand tear (*2*) were determined. In addition to tabulating the time to reach maximum values the time to reach various percentages (60, 70, 75, 80, 85, 90) of maximum values was determined for tensile strength and also for the other physicals.

We have defined state of cure as the ratio of the physical property at a given cure divided by the maximum value of the physical property. It was therefore thought of interest to see if the state of cure for any given cure, such as 15 minutes, could offer a useful index of rate of cure. For this purpose the ratio of tensile at all undercures to maximum tensile was determined and similar ratios for tensile product, rebound, modulus, and durometer. (In the case of modulus and durometer the 180-minute cure was taken as the maximum value.) Since the rebound of the uncured stock is appreciable, the ratio of the increase in rebound over uncured rebound at all undercures to the increase in rebound over uncured rebound for the maximum rebound was also calculated.

Finally, it was thought, the slope of the curve for the various physical properties *vs.* cure time at marked undercures might furnish an index of cure. Therefore the difference in tensile for various undercures—e.g., tensile at 15 minutes minus tensile at 8 minutes—divided by maximum tensile was tabulated and similar ratios for tensile product, tear, and rebound.

Inasmuch as the T-50 test is probably the most generally accepted index of rate of cure for natural rubber, the indexes enumerated above were tested by determining their correlation with T-50. The tensile ratio which for this series of stocks was taken as the tensile at 15 minutes divided by the maximum tensile was found to give the best correlation.

The results are summarized in Figures 3 and 4, where the tensile ratio and other indexes of rate of cure are plotted against T-50 at the 60-minute cure. The other indexes are those found to give the closest correlation with T-50 or which have been previously suggested in the literature.

The prediction indexes (P.I.) indicating the degree of correlation between the various indexes of rate of cure and T-50 are listed on the figures. (Prediction index $= 1 - \sqrt{1 - r^2}$, where r is the coefficient of correlation calculated according to standard statistical methods. P.I. is 0 for no correlation and 1 for perfect correlation. For six pairs of values as in the present instance $r > 0.8$ or P.I. > 0.4 shows significant correlation within the 95% confidence limits.) The prediction index of 0.84 for tensile ratio was the highest found. Likewise the fact that tensile ratio is the only function which can be fairly well approximated by a single straight line confirms the indication given by the prediction indexes.

The tensile ratio has been used in the authors' laboratory as an index of rate of cure for various channel black stocks. In a series of twenty-seven experimental blacks in the same base formula used for the pigments in Table I the tensile ratio and time to reach maximum tensile were both measured. The rate of cure as defined previously was then determined by dividing the tensile ratio of each experimental stock by the tensile ratio for the standard control which was cured at the same time as the experimental sample. The same control was, of course, used throughout the series. Likewise, the rate of cure was also estimated by dividing the time to reach maximum tensile of the control by the time to reach maximum tensile for the sample. The

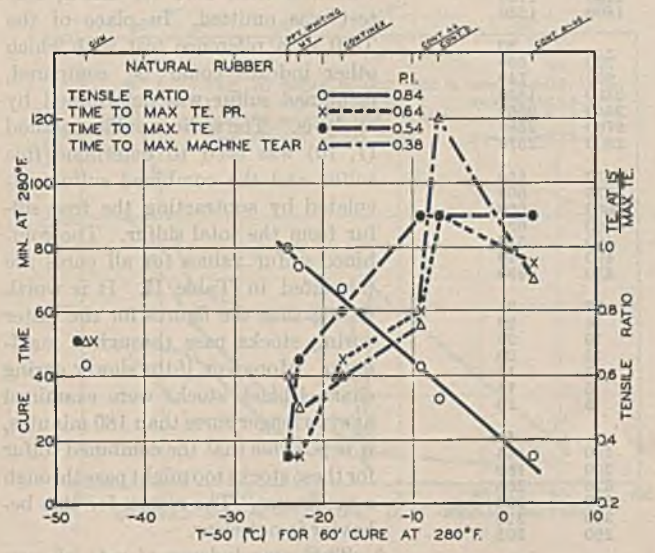

Figure 3

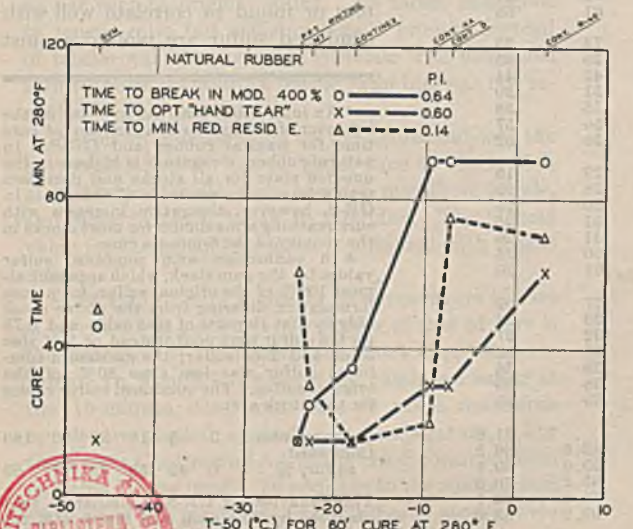

Figure 4

Table II. GR-S

Formula		Gum	Pptd. Whiting	MT	Continex	HMF	Continental AA	Continental D	Continental R-40
GR-S		100	100	100	100	100	100	100	100
Zinc oxide		5	5	5	5	5	5	5	5
Coal-tar softener		5	5	5	5	5	5	5	5
Mercaptobenzothiazole		1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sulfur		2	2	2	2	2	2	2	2
Precipitated whiting		...	78	....	....	....	....	....	....
Medium thermal (MT)		...	...	50	....	....	....	....	....
Continex (SRF)		...	...	....	50	....	....	....	....
High modulus furnace (HMF)		...	...	....	....	50	....	....	....
Continental AA (EPC)		...	...	....	....	....	50	....	....
Continental D (MPC)		...	...	....	....	....	....	50	....
Continental R-40 (CC)		...	...	....	....	....	....	....	50
					Cure at 307° F.				
	Min.								
Modulus at 300% elongation, lb./sq. in.	0	...	....	....	....	....	....	....	....
	8	...	240	90	220	210	200	260	310
	15	...	240	210	580	790	510	360	390
	30	...	240	320	850	1270	900	800	410
	60	...	240	400	1130	1420	1230	1180	970
	90	...	240	410	1130	1450	1320	1260	1100
	180	...	250	420	1190	1470	1480	1500	1250
Tensile at break, lb./sq. in.	0	0	0	0	40	50	100	....	80
	8	160	700	520	650	560	300	720	500
	15	190	790	940	1340	1880	1570	950	740
	30	190	760	1130	1630	2200	2380	2320	1820
	60	190	760	1010	1550	2200	2620	2550	2360
	90	190	760	1010	1460	2170	2590	2760	2580
	180	190	500	840	1400	2100	2570	2600	2570
Elongation at break[a], %	0	460	300	275	415	410	490	550	550
	8	315	590	880	685	600	640	675	600
	15	400	560	805	590	550	625	660	660
	30	300	550	665	520	450	575	600	650
	60	315	550	625	390	415	465	490	550
	90	340	545	590	385	395	450	490	540
	180	225	455	520	350	385	425	430	480
Breaking set, %	0	...	12	15	17	15	32	37	31
	8	...	10	10	7	10	22	16	29
	15	...	4	9	4	9	17	19	26
	30	...	6	6	5	5	12	15	20
	60	...	4	5	4	4	5	5	15
	90	...	5	5	4	2	5	5	14
	180	...	5	4	4	2	5	5	10
Tear, lb./in.	0	...	20	20	....	20	30	....	55
	8	...	70	115	155	125	50	100	145
	15	...	90	135	225	265	230	200	180
	30	...	90	100	185	225	325	320	265
	60	...	85	100	155	220	285	380	280
	90	...	80	110	150	175	265	380	315
	180	...	80	85	150	170	270	290	305
Durometer at 25° C., 30-second reading	0	10	15	14	15	16	21	23	33
	8	33	42	28	33	33	38	37	41
	15	36	44	35	43	44	45	45	47
	30	35	44	41	49	51	55	54	57
	60	35	45	42	51	52	59	58	61
	90	35	43	42	52	55	58	58	61
	180	35	43	43	51	54	60	61	66
Durometer at 100° C., 30-second reading	0	8	11	6	9	11	11	14	23
	8	32	44	33	33	33	29	29	35
	15	35	44	36	42	44	40	42	44
	30	35	44	41	48	50	50	51	50
	60	34	43	41	51	51	56	55	58
	90	34	41	41	54	53	55	56	57
	180	35	41	40	52	51	57	59	62
Rebound at 25° C., %, Bashore	0	43	28	33	20	28	25	23	19
	8	53	39	44	38	38	30	28	22
	15	51	40	47	40	41	31	30	25
	30	52	40	48	43	42	34	31	25
	60	53	39	46	43	43	35	31	26
	90	53	40	46	42	42	33	30	26
	180	52	38	46	41	42	32	31	26
Rebound at 100° C., %, Bashore	0	26	33	35	32	30	30	27	22
	8	56	48	48	40	40	32	29	24
	15	57	48	50	45	43	33	32	27
	30	58	49	57	51	48	39	38	32
	60	60	47	55	53	49	40	39	34
	90	59	48	54	52	51	40	38	35
	180	58	46	54	51	50	39	39	35
Combined sulfur, % of original sulfur[b]	8	70.5	89.5	43.3	45.5	37.5	32.6	....	31.6
	15	84.8	90.6	64.0	65.6	66.3	54.2	43.6	36.2
	30	96.9	...	78.4	....	83.2	72.4	59.0	50.1
	60	98.4	95.0	86.5	85.6	....	87.4	85.2	70.0
	90	98.8	90.8	90.0	....	....	89.2	89.9	82.1
	180	98.0	72.5	70.0	69.8	77.6	90.4	93.9	88.0

prediction index indicating the correlation between the rate of cure determined in these two ways was then found to be 0.24. Since for twenty-seven pairs of values a prediction index greater than 0.08 shows a significant correlation, there is apparently a definite relation between these two methods of determining rate of cure. Of the two methods, tensile ratio can be determined more precisely.

DETERMINATION OF RATE OF CURE IN GR-S

As in the case of natural rubber various pigments were studied in a typical formulation, 26 volumes of pigment per 100 volumes of polymer being used as before. The formula and test results appear in Table II. Since T-50 does not show much variation with cure for GR-S, this test was omitted. In place of the T-50 as a reference test with which other indexes could be compared, combined sulfur was determined by analysis. The sodium sulfite method (*1, 10*) was used to determine free sulfur and the combined sulfur calculated by subtracting the free sulfur from the total sulfur. The combined sulfur values for all cures are tabulated in Table II. It is worth noting that the figures for the faster curing stocks pass through a maximum. Moreover, if the slower curing channel black stocks were examined at even longer cures than 180 minutes, it is possible that the combined sulfur for these stocks too might pass through a maximum. The reason for this behavior is not clear.

The same indexes of rate of cure studied in natural rubber were determined. In Figures 5 and 6 those indexes suggested by other investigators or found to correlate well with combined sulfur are plotted against

[a] An interesting contrast appears in the behavior of elongation as a function of cure time for natural rubber and GR-S. In natural rubber, elongation is highest in the uncured state for all stocks and decreases regularly as cure proceeds (Table I). In GR-S, however, elongation increases with cure reaching a maximum for most stocks in the vicinity of the 8-minute cure.

[b] In connection with combined sulfur values for the gum stock, which approach almost 100% of the original sulfur, for a gum formulation differing from the above stock only in that 10 parts of zinc oxide and 0.75 part of sulfur were used instead of 5 of zinc oxide and 2 of sulfur, the maximum combined sulfur was less than 80% of the original sulfur. The combined sulfur values for this stock are:

Cure, minutes	0	8	15	30	90	180
Combined sulfur, %	0	42	58	69	77	63

This behavior for GR-S is similar to that observed by Thornhill and Smith (*13*) for natural rubber. They found that for higher sulfur loading the per cent of the original sulfur combined at a given cure is higher.

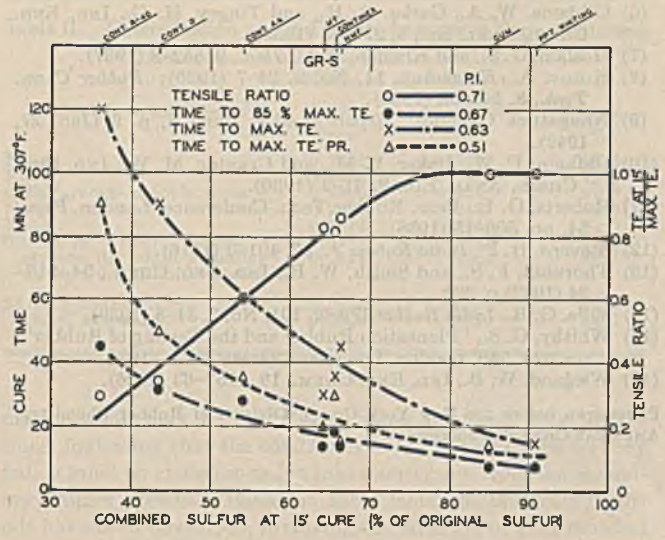

Figure 5

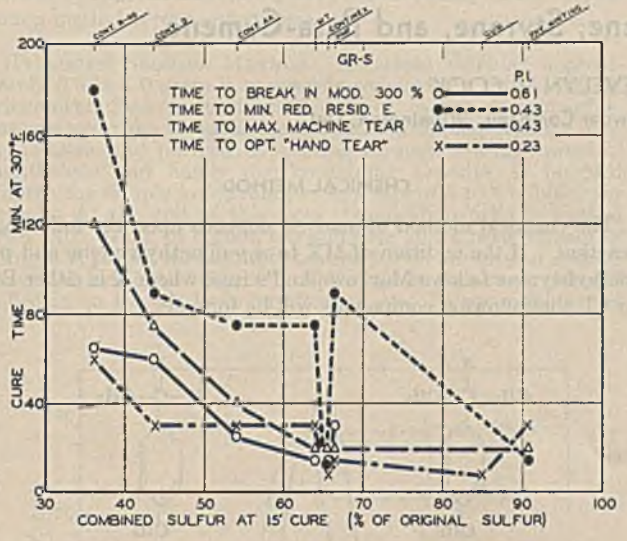

Figure 6

the combined sulfur at the 15-minute cure expressed as per cent of original sulfur. An index analogous to tensile ratio but utilizing tensile product instead of tensile was also found to correlate with combined sulfur almost as well as tensile ratio but was not included in the graph.

Prediction indexes listed on the figures indicate the best correlation for tensile ratio, time to reach 85% of maximum tensile, time to reach maximum tensile, and time to reach break in modulus. The highest value for the prediction index is obtained with tensile ratio.

Since rate of cure increases as temperature of cure is increased, a good test of an index of rate of cure is the behavior of the index at different cure temperatures. In Figure 7, tensile ratio, combined sulfur at the 15-minute cure, and time to reach maximum tensile are plotted against cure temperature. For these tests Continental AA in the GR-S formula given in Table II was used. In addition to the stocks cured at various temperatures, four duplicate stocks were cured at 307° F. in order to give some indication of the reproducibility of these three methods.

Prediction indexes giving the correlation of the indexes of cure rate with cure temperature are listed at the top of Figure 7. The best correlation with cure temperature is obtained with tensile ratio and combined sulfur. Time to reach maximum tensile also shows a significant correlation with cure temperature but does not correlate as closely as the other two indexes. (For the eight stocks used in this case a prediction index > 0.3 or a correlation coefficient > 0.7 indicates a significant correlation within 95% confidence limits.)

DISCUSSION

Studies with GR-I stocks also indicate that the tensile ratio is a useful index of cure for this synthetic. It is planned to present these results in a separate paper.

In dealing with certain special problems the time required to obtain a satisfactory index of rate of cure may possibly be shortened. For example, in comparing a series of stocks all of which have very similar maximum tensiles, the tensile of the proper undercure is approximately proportional to the tensile ratio and can be used similarly as an index of rate of cure. A case of this sort would occur in the control testing of rubber channel blacks or other pigments. On the other hand, although the maximum tensiles for different stocks in a series may be different, the cure rate may be sufficiently similar so that the tensile of a fixed cure in the maximum tensile range, say the 60-minute cure, may be fairly close to the maximum tensile for all stocks. In this event the tensile ratio would be approximately equal to the tensile at 15 minutes/tensile at 60 minutes, which could then be used as an index of rate of cure and would only require the making of two cures. A possible example of this type might be a series of stocks containing different amounts of petroleum-type softeners.

The rate-of-cure results for both natural rubber and GR-S indicate that the tensile ratio is a satisfactory index of rate of cure even in a series of stocks containing a widely different variety of pigments. However, it seems likely that the greatest usefulness for the tensile ratio will occur in the comparison of similar pigments or other compounding ingredients such as carbon blacks, inorganic pigments, softeners, antioxidants, etc., in the same test formula.

The above short cuts as well as tensile ratio itself are satisfactory indexes of rate of cure only within a set of stocks milled and cured side by side, such as the stocks in Tables I and II. In the comparison of stocks not milled and cured together a reference control is customarily run at the same time as each stock. The

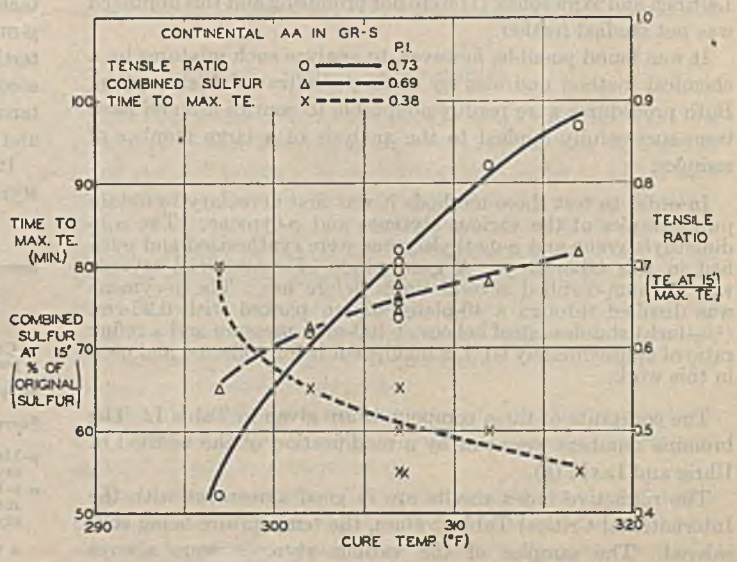

Figure 7

tensile ratio of the individual stocks divided by the tensile ratio for the corresponding control should then be taken as the index of rate of cure.

ACKNOWLEDGMENT

The authors wish to thank Miss B. C. Myerson and Miss M. Sohn for their assistance in obtaining the experimental data.

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Determination of Alpha,Para-Dimethylstyrene

In the Presence of Para-Methylstyrene, Styrene, and Para-Cymene

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α,*p*-Dimethylstyrene can be determined in the presence of *p*-methylstyrene, styrene, and *p*-cymene by two independent methods. The chemical method depends upon addition of hydrogen chloride or bromide to the styrenes with subsequent estimation of the tertiary halide formed by α,*p*-dimethylstyrene. The other method involves an analysis of the ultraviolet absorption curve of such mixtures. Results obtained by these independent methods are in good agreement.

NEED has recently arisen for a method for the determination of α,*p*-dimethylstyrene in the presence of *p*-methylstyrene, styrene, and *p*-cymene. Since no satisfactory procedures were available for the analysis of such mixtures, chemical methods, ultraviolet absorption, and polarographic analysis were considered.

Preliminary experiments on the last, using the technique of Laitinen and Wawzonek (*1*) were not promising and this approach was not studied further.

It was found possible, however, to analyze such mixtures by a chemical method and also by means of ultraviolet absorption. Both procedures were readily adaptable to control use and have been successfully applied to the analysis of a large number of samples.

In order to test these methods it was first necessary to obtain pure samples of the various styrenes and *p*-cymene. The α,*p*-dimethylstyrene and *p*-methylstyrene were synthesized and purified in this laboratory. A good grade of commercial styrene was vacuum-distilled several times before use. The *p*-cymene was distilled through a 40-plate column packed with 0.23-cm. ($^3/_{32}$-inch) stainless steel helices at 100-mm. pressure and a reflux ratio of approximately 60/1, a middle cut being collected and used in this work.

The constants of these compounds are given in Table I. The bromine numbers were run by a modification of the method of Uhrig and Levin (*9*).

The refractive index results are in good agreement with the International Critical Tables values, the temperature being considered. The samples of the various styrenes were always freshly vacuum-distilled before being used.

CHEMICAL METHOD

The chemical method of analysis depends upon the following reaction. If the addition of HX to α,*p*-dimethylstyrene and *p*-methylstyrene follows Markownikoff's rule, where X is either Br or Cl, the following compounds will be formed:

(1) (2)

The halogen in (1), formed from α,*p*-dimethylstyrene, is attached to a tertiary carbon atom, while that in (2), formed from *p*-methylstyrene, is attached to a secondary carbon atom. The tertiary halogen in (1) is much more readily hydrolyzed than the secondary in (2), and this fact offers a means of analytically determining α,*p*-dimethylstyrene in the presence of *p*-methylstyrene and styrene. There is no reaction of HX with *p*-cymene.

It has been reported that hydrogen bromide adds rapidly to styrene (*2*, *10*), and that the secondary bromide formed by nor-

Table I. Constants of Materials Used in Testing Methods

Compound	Formula	$n_D^{25.5}$	n_D (I.C.T.)	Bromine No. Observed	Bromine No. Theory
p-Cymene	*p*-$CH_3.C_6H_4.CH(CH_3)_2$	1.4913	1.4947 (15° C.)	<1	0
Styrene	$C_6H_5.CH:CH_2$	1.5467	1.5467 (20° C.)[a]	151	154
p-Methylstyrene	*p*-$CH_3.C_6H_4.CH:CH_2$	1.5421	1.5447 (16.4° C.)	137	135
α,*p*-Dimethylstyrene	*p*-$CH_3.C_6H_4.C(CH_3):CH_2$	1.5356	1.5344 (18.7° C.)	117	121

[a] Value of Moore, Burk, and Lankelma (*7*).

Table II. Determination of α,p-Dimethylstyrene in Known Mixtures

(Using method involving addition of HBr)

Other Materials Present	Weight of α,p-Dimethylstyrene Present	Recovered	Recovery
	Gram	*Gram*	%
None	0.2731	0.2545	93.3
None	0.2131	0.1977	92.8
0.2080 gram p-methylstyrene	0.2408	0.2215	91.9
None	0.2693	0.2477	92.0
None	0.2414	0.2245	94.0
0.1 ml. of benzene, 0.2 ml. of styrene, 0.2 ml. of p-methylstyrene, 0.5 ml. of p-cymene	0.4147 0.2613	0.3942 0.2522	95.2 96.7
25.6% p-methylstyrene and 48.8% p-cymene	0.0854 0.1006	0.0796 0.0926	94.2 92.0

mal addition may be hydrolyzed under relatively mild conditions, indicating that the conditions of hydrolysis of the tertiary halide must be chosen so as to avoid interference from the secondary halides present. Based on these facts, the following methods have been developed, involving the addition of HX, removal of excess, followed by hydrolysis, and estimation of the tertiary halide formed from α,p-dimethylstyrene under conditions where there is no appreciable hydrolysis of the secondary halide formed from p-methylstyrene and styrene.

Hydrogen Bromide Method. A sample weighing approximately 0.5 to 1.0 gram is accurately weighed out into a 125-ml. Erlenmeyer flask containing 25 ml. of carbon tetrachloride. Gaseous hydrogen bromide, generated by dropping bromine onto naphthalene and purified by passing through several towers of naphthalene and finally one containing Drierite, is bubbled through the sample for 30 minutes at a rate of 3 to 4 bubbles per second. At the end of this time the addition tube is washed down with 10 ml. of carbon tetrachloride and a brisk stream of nitrogen is passed through the solution for 30 minutes to remove unreacted hydrogen bromide. The flask is then removed and chilled in an ice-salt bath for 10 minutes. Thirty milliliters of previously chilled 90% alcohol are then added, the flask is swirled for a few seconds, two drops of methyl red indicator are added, and the cold solution is rapidly titrated with standard 0.1*N* alcoholic potassium hydroxide to a bright yellow end point lasting for 10 seconds. Table II gives the results obtained by this method on known samples.

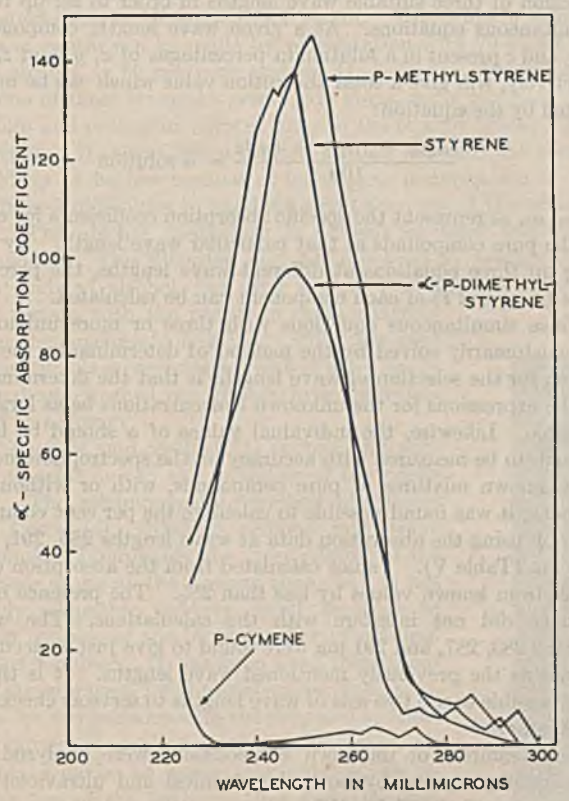

Figure 1. Ultraviolet Absorption Curves

For α, p-dimethylstyrene, p-methylstyrene, styrene, and p-cymene in ethanol solutions

Hydrogen Chloride Method. The procedure is the same as that used in the hydrogen bromide method with the following changes:

Benzene instead of carbon tetrachloride is used as the solvent.

Forty milliliters of 80% alcohol are used to effect hydrolysis of the tertiary halide.

The titration is performed at room temperature and the sample titrated with 0.1*N* alcoholic potassium hydroxide to a 30-second bright yellow end point. Hydrogen chloride was generated by dropping concentrated hydrochloric acid into concentrated sulfuric acid. The evolved gas was passed through a tower filled with concentrated sulfuric acid before being passed into the solution being analyzed.

Table III gives the results obtained by this method on known samples. The end points are considerably sharper than those given by the hydrogen bromide method.

Table III. Determination of α,p-Dimethylstyrene in Known Mixtures

(Using method involving addition of HCl)

Substances Present	Weight of α,p-Dimethylstyrene Present	Recovered	Recovery
	Gram	*Gram*	%
p-Cymene	None	None	...
Styrene	None	None	...
p-Methylstyrene	None	None	...
α,p-Dimethylstyrene	0.2936	0.2740	93.5
	0.4739	0.4508	95.3
	0.4116	0.3903	94.7
	0.3077	0.2940	95.5[a]
	0.5693	0.5415	95.3[a]
α,p-Dimethylstyrene plus 0.5 ml. each of p-cymene, styrene, and p-methylstyrene	0.4015 0.4052	0.3855 0.3902	96.0 96.2

[a] HCl gas added for 1 hour instead of 30 minutes as in all other cases.

Table IV. Analyses by Hydrogen Bromide and Hydrogen Chloride Methods

Sample	α,p-Dimethylstyrene HBr method	HCl method
	%	%
A	19.6, 20.2	19.8, 20.6
B	29.2, 29.5	28.2, 29.3
C	20.2, 19.9	20.2, 19.8
D	20.5, 20.4	20.6, 20.6
E	22.6, 22.0	22.6, 23.3
F	10.6, 10.7	9.5, 9.5
G	32.8, 32.6	32.2, 32.0
H	32.9, 32.6	31.8, 32.5

DISCUSSION OF RESULTS

An examination of Tables II and III shows that somewhat low recoveries (about 95%) of α,p-dimethylstyrene are obtained in every case. Therefore, to correct for this a factor of 1.05 is used in calculating the results. This low recovery of α,p-dimethylstyrene is not raised by a longer time of addition of the hydrogen halide. It may be due to the fact that a small fraction of the addition takes place contrary to Markownikoff's rule. A consideration of the results obtained by use of these methods in the analysis of over fifty samples of known and unknown composition indicates that the precision of the methods is approximately ±3% of the α,p-dimethylstyrene present.

Table IV shows the agreement between the hydrogen bromide and hydrogen chloride methods in the analysis of eight different samples of unknown composition.

The agreement between the results obtained by the two methods is seen to be good. Since the hydrogen chloride method is somewhat easier to use and the end point is sharper, it is recommended for use.

ULTRAVIOLET ABSORPTION METHOD

The spectrophotometric method has been successfully applied to the quantitative determination of compounds having characteristic absorption bands in the ultraviolet region of the spectrum. Good examples of this method used in calculating two, three, and four constituents of a mixture have been reported in the literature (*3–6*).

In mixtures of pure styrene, α,*p*-dimethylstyrene, *p*-methylstyrene, and *p*-cymene it was found possible to determine the amount of each styrene present within 2% of the known value.

The absorption data were obtained from measurements made with a Beckman quartz spectrophotometer. The solvent was ethanol in all cases. The formulas used in making the calulations use the term specific α:

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

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

The samples of pure α,*p*-dimethylstyrene, *p*-methylstyrene, styrene, and *p*-cymene used to obtain the specific absorption coefficients of the pure compounds were the same as those used in the development of the chemical method previously described.

The values at different wave lengths are shown in Figure 1. The curve for styrene agrees very closely with previously published data concerning this compound (*8*).

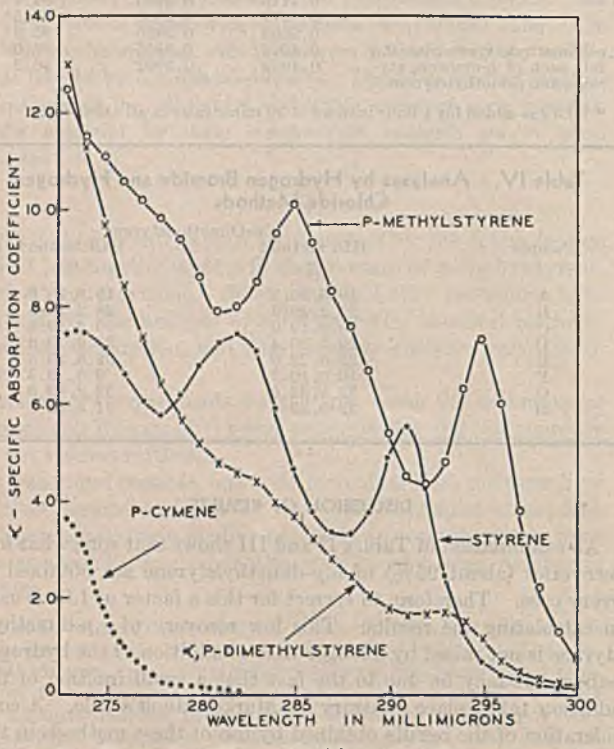

Figure 2. Ultraviolet Absorption Curves
For α, *p*-dimethylstyrene, *p*-methylstyrene, styrene and *p*-cymene in ethanol solutions (enlarged scale of spectral region 275 to 300 mμ)

The styrenes have a strong absorption band in the spectral region 248 to 252 mμ with less pronounced but yet distinct bands in the region to 300 mμ. For the three styrenes the bands in the region 248 to 252 mμ are so similar that no attempt was made to use them for quantitative determinations. A larger scale graph of the region 280 to 300 mμ (Figure 2), shows that at certain wave lengths there are sufficient differences in α of the pure compounds to permit quantitative calculations. It is also evident that *p*-cymene has its absorption values below 280 mμ and therefore does not appreciably affect absorption values above 280 mμ, even though present in quantities up to 70 or 80%.

Table V. Per Cent Composition

Mixture	Composition of Mixture of Pure Compounds	Known	Calculated from Ultraviolet Absorption Data	Error
		%	%	%
1	α,*p*-Dimethylstyrene	32	30.8	1.2
	p-Methylstyrene	42	42.4	0.4
	Styrene	26	26.0	0
2	α,*p*-Dimethylstyrene	5	6.0	1.0
	p-Methylstyrene	5	5.3	0.3
	Styrene	90	88.1	1.9
3	α,*p*-Dimethylstyrene	5	3.9	1.1
	p-Methylstyrene	90	90.3	0.3
	Styrene	5	5.3	0.3
4	α,*p*-Dimethylstyrene	25	24.3	0.7
	p-Methylstyrene	26	26.3	0.3
	Styrene	25	25.9	0.9
	p-Cymene	24	...	...
5	α,*p*-Dimethylstyrene	20	21.9	1.9
	p-Methylstyrene	30	29.4	0.6
	Styrene	35	35.8	0.8
	p-Cymene	15	...	...

Table VI. Samples of Unknown Composition

Sample	α,*p*-Dimethylstyrene	
	Chemical method	Ultraviolet absorption method
	%	%
1	23.0, 22.9	21.7, 22.3
2	20.0, 20.2	19.8, 20.3
3	32.5, 32.3	32.8, 32.5
4	27.3, 27.4	27.8, 27.5

Quantitative determinations on three unknowns require the selection of three suitable wave lengths in order to set up three simultaneous equations. At a given wave length, components *a*, *b*, and *c* present in a solution in percentages of x, y, and z, respectively, will give a total absorption value which can be represented by the equation:

$$\frac{(\alpha_a)x + (\alpha_b)y + (\alpha_c)z}{100} = \alpha \text{ solution}$$

α_a, α_b, α_c represent the specific absorption coefficients for each of the pure compounds at that particular wave length. By setting up three equations at different wave lengths, the percentages (x, y, and z) of each component can be calculated.

Since simultaneous equations with three or more unknowns are customarily solved by the method of determinants, the criterion for the selection of wave lengths is that the determinants in the expressions for the unknown concentrations be as large as possible. Likewise, the individual values of α should be large enough to be measured with accuracy on the spectrophotometer.

In known mixtures of pure compounds, with or without *p*-cymene, it was found possible to calculate the per cent composition by using the absorption data at wave lengths 285, 291, and 295 mμ (Table V). Values calculated from the absorption data differ from known values by less than 2%. The presence of *p*-cymene did not interfere with the calculations. The wave lengths 283, 287, and 291 mμ were found to give just as accurate values as the previously mentioned wave lengths. It is therefore possible to use two sets of wave lengths to serve as checks on each other.

Four samples of unknown compositions were analyzed for α,*p*-dimethylstyrene by both the chemical and ultraviolet absorption methods (Table VI).

The agreement between these independent methods is seen to be good.

ACKNOWLEDGMENTS

The authors wish to express their thanks for the assistance in running analyses given by Jordan P. Snyder and Miss Mary Thomas Montgomery.

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Determination of Soluble Pectin and Pectic Acid by Electrodeposition

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In a new approach to the determination of pectin, solutions of pectin or pectates are deashed by the use of ion-exchange resins. The pectin or pectic acid is electrolytically deposited at a platinum anode in a weighable form. The method requires less of the analyst's time and attention than does the calcium pectate method and, with partially purified solutions, gives results of the same order of accuracy. The method is especially applicable to amounts of pectin ranging between 5 and 50 mg.

INVESTIGATIONS designed to develop new and extended uses for pectin have made desirable an analytical method capable of determining small amounts of pectin. Approximate estimations of pectin can be made by precipitation with 70 to 80 per cent alcohol or 50 per cent acetone (*1*, *3*, *8*, *9*). Alcohol and acetone of these strengths precipitate gums, some proteins, and calcium and potassium salts of some of the organic acids as well as pectin. However, when dilute solutions are used the results are likely to be low because of incomplete precipitation of the pectin and difficulties in handling small amounts of the precipitate. The alcohol precipitate is difficult to filter and wash thoroughly. This filtration may be improved by precipitation with acidified alcohol, but after several washings enough acid may remain with the pectin to cause charring when the precipitate is dried. Fifty per cent acetone yields a precipitate which is more easily filtered and washed but affords little improvement in accuracy.

The pectic acid method (*1*) is long and subject to error due to the solubility of the pectic acid. The calcium pectate method of Emmet and Carré (*4*), although tedious and time-consuming, is probably the most reliable of the methods in use at the present time, but is not readily adaptable to very small amounts of pectin because of the large number of manipulations required. It has been used throughout this investigation for the analysis of stock solutions for comparisons with the proposed method. The proposed method is the result of an attempt to devise an accurate method which requires less of the analyst's time and attention and can be used to determine smaller amounts of pectin than the method of Emmet and Carré.

Since the soluble pectinous materials are negatively charged colloids, it was decided to investigate the possibility of collecting the pectin on the anode of a suitably arranged electrolysis system. Brown (*2*) unsuccessfully attempted to precipitate pectin electrolytically from aqueous solutions containing large amounts of electrolytes. Griggs and Johnstin (*5*) observed flocculation of pectin at the anode on electrolysis with 110-volt direct current. The authors have found that pectin can be quantitatively determined by electrodeposition, provided the electrolyte concentration of the solution is low. Ion-exchange resins can be used when it is necessary to remove the electrolytes before electrodeposition.

APPARATUS

A conventional electrolysis apparatus supplied with 220-volt direct current from a rectifier-transformer unit was used. A mercury cathode cell was constructed from a 250-ml. Griffin-type beaker, into the side of which was fused a platinum wire (Figure 1). A mercury-filled side arm was added to provide flexibility in the connection to the negative binding post. Clean mercury completely covering the platinum wire in the vessel served as the cathode. The anode was a disk of 45-mesh platinum gauze 6.25 cm. (2.5 inches) in diameter, edged with 0.075-cm. (0.03-inch) platinum wire to give rigidity and supported by a 15-cm. (6-inch) piece of 0.127-cm. (0.05-inch) platinum wire attached to the center (Figure 1). Similar electrodes are avail-

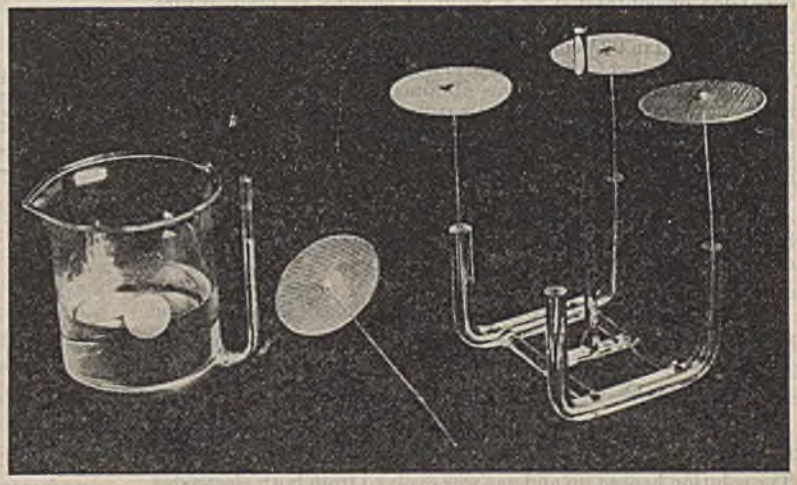

Figure 1. Electrolysis Vessel, Anode and Anodes in Holder

Table I. Electrodeposition of Pectin from Solutions Containing Various Percentages of Alcohol

(25-ml. aliquots of stock solution. Time, 5 hours)

Alcohol Content	Weights of Deposits				Remarks
% by volume	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
30	17.2	18.9	20.6	..	Solutions became hot. One of 4 deposits did not adhere to anode
40	21.8	22.1	22.2	21.9	Compact deposits
50	7.6	7.9	8.5	5.7	Deposits not compact
60	7.1	7.6	9.2	9.8	Bulky deposits

Table II. Time Required for Electrodeposition of Pectin from 40 Per Cent Alcohol Solutions

(25-ml. aliquots of stock solution)

Time	Weights of Deposits				Average	Remarks
Hr.	*Mg.*	*Mg.*	*Mg.*	*Mg.*		
2	19.9	20.4	19.4	19.0	19.7	Precision poor
3	18.9	20.5	19.6	20.3	19.8	Precision poor
4	20.8	20.3	20.1	20.1	20.3	Precision fair
5	21.8	22.1	22.2	21.9	22.0	Precision good
6	21.9	21.7	22.2	22.1	22.0	Precision good

able from platinum manufacturers. This electrode system has proved to be much more satisfactory than one using either cylindrical or spiral electrodes, since the mercury cathode–disk anode system makes it possible to have all the solution under the influence of the field without stirring.

Ion-exchange resin columns were prepared from 24 × 190 mm. glass tubing constricted to a 6-mm. outlet (Figure 2). Ten grams drained weight of ion-exchange resin, supported on a glass wool mat, was used in each tube. The cation-exchange column was mounted so that the effluent dripped directly into the acid-absorbing bed.

ELECTRODEPOSITION OF PECTIN FROM LOW-ASH SOLUTIONS

The following procedure was found to give a firm deposit that adhered very well to the anode:

An aliquot of the aqueous solution containing approximately 5 to 50 mg. of pectin was placed in the electrolysis vessel and diluted to 58 ml. with distilled water, and 42 ml. of 95 per cent ethyl alcohol were added with stirring. To obtain quantitative deposition in the shortest time, the alcohol concentration must be close to 40 per cent by volume, as indicated in Table I. Precipitation of the pectin at this point indicates that the electrolyte content is too high and that the solution must be deashed. During the electrolysis the vessel was immersed in a water-ice bath to prevent an undue rise in the temperature of the solution. The anode was lowered into the solution so that the gauze was barely covered and 220-volt direct current was applied. The current varied between 5 and 20 milliamperes, depending on the electrolyte content and the temperature of the solution. No stirring of any kind was employed during the run. Occasionally it was necessary to adjust the anode level if the solution had expanded or contracted as a result of changes in temperature. At the end of 5 hours the anode was withdrawn from the vessel and immersed in 99 per cent ethyl alcohol for 3 minutes and then in anhydrous ether for 3 minutes.

A very compact deposit was obtained, which was further dehydrated by the alcohol-ether treatment to a very thin uniform film. This alcohol-ether treatment permitted complete drying in one hour at 105° C. The electrodes were cleaned by immersion in boiling water for a few minutes, followed by rinsing in distilled water. If the deposit was pectic acid, the addition of a little dilute alkali aided in the solubilization. Removal of the deposit by ignition in a flame was avoided because of the possible danger of changing the electrode surface.

Table II shows the relation between the time of deposition and the weight of the deposit.

REMOVAL OF ELECTROLYTES FROM HIGH-ASH SOLUTIONS

Pectin deposition from extracts of fruit was unsuccessful because the current was transferred by the electrolytes present. The solution heated up and gas was evolved from both electrodes. No deposit was obtained.

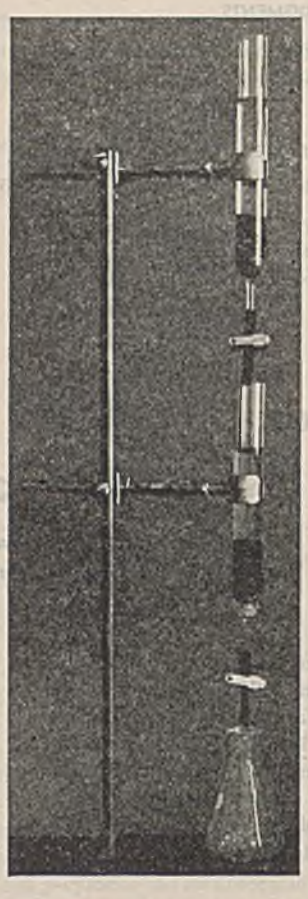

Figure 2. Deashing System

Upper, cation exchange column; lower, acid adsorbing column

A system of commercial ion-exchange resins has been used to remove electrolytes from pectin solutions in this laboratory. (The use of exchange materials for the removal of ash constituents from pectin solutions was first investigated in this laboratory by W. D. Maclay and co-workers who are investigating the efficiencies of various commercial products for this purpose.) The IR 100 resin removes the cations by exchange with hydrogen ion, while the IR 4 resin removes the acids which would interfere, by conductance, with the electrodeposition of pectin. No pectin was lost during this process when the conditions described below were followed. The capacity and regeneration of these resins are described by the manufacturer (7).

Solutions containing 0.4 to 1.0 mg. of pectin per ml. were deashed in the following manner: The columns were rinsed with two 10- to 15-ml. portions of the solution, with thorough drainage between rinses. The rinsings were discarded and the main portion of the solution was deashed by percolation through the columns at the rate of approximately 3 ml. per minute.

The pectin concentration of the solution was unchanged by this treatment (Table III). The pectin solutions treated in this manner were low in ash, ranging from 0.5 to 4 mg. per 100 ml., and successful electrodepositions could be made from such solutions. The resin system has been used successfully on neutral, acid, and alkaline aqueous extracts of pectin.

Table III. Pectin Content of Solutions before and after Ion-Exchange Treatment

Source of Pectin	Before[a]	After[a]
	Mg./ml.	*Mg./ml.*
Extract of whole grapefruit	1.03	1.03
Extract of whole orange	0.64	0.64
Extract of whole apple	0.41	0.40
High-ash commercial pectin	0.35	0.35

[a] Average of three analyses by method of Emmet and Carré (4).

Table IV. Electrodeposition of Pectin from Acid Extracts of Whole Fruit after Removal of Electrolytes[a]

Extract of Whole	Aliquot of Original Solution	Pectin Deposited				Average
	Ml.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg./ml.*
Grapefruit	2.5	14.2	14.1	14.1	...	5.65
	5.0	28.4	28.1	28.2	28.0	5.64
	10.0	57.8	57.1	58.3	57.7	5.77
Orange	5.0	13.8	14.4	13.7	13.8	2.78
	10.0	29.0	28.9	28.7	28.5	2.88
Apple	5.0	10.9	11.1	11.1	11.1	2.22
	10.0	21.4	21.4	21.8	22.0	2.17
	25.0	55.2	54.8	56.7	56.2	2.23

[a] Averages of triplicate analyses by method of Emmet and Carré (4) yielded 5.16 mg. of pectin per ml. of grapefruit extract, 2.57 mg. per ml. of orange extract, and 1.99 mg. per ml. of apple extract.

Table V. Electrodeposition of Pectic Acid from Extracts of Whole Apple and Grapefruit after Acetone Purification, Hydrolysis, and Removal of Electrolytes[a]

Extract of Whole	Aliquot of Original Solution	Pectin Acid Deposited				Average
	Ml.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg./ml.*
Apple	10.0	10.7	10.6	10.7	10.7	1.07
	20.0	22.1	22.1	22.1		1.10
Grapefruit	5.0	10.6	10.4	10.7	10.7	2.12
	10.0	21.1	21.0	21.5	21.2	2.12

[a] Averages of triplicate analyses by method of Emmet and Carré (*4*) yielded 1.07 mg. of pectic acid per ml. of apple extract and 2.13 mg. of pectic acid per ml. of grapefruit extract.

ANALYSES OF EXTRACTS OF GRAPEFRUIT, ORANGES, AND APPLES

The flavedo was removed from oranges and grapefruit, leaving most of the albedo intact. The apples were not peeled. The following modification of the extraction procedure of Myers and Baker (*6*) was used:

The fruit was cut up and disintegrated in a Waring Blendor with the addition of sufficient water and hydrochloric acid to give a thin slurry of pH 1.7 to 2.0. The slurry was then heated in a water bath at 85° C. for 30 minutes and filtered through analytical grade Celite. The residual pulp was extracted and filtered twice more in the same manner. The clear extracts were combined and diluted, if necessary, to a pectin content of approximately 1 mg. per ml. Aliquots of 100 ml. were taken for analysis by the calcium pectate method of Emmet and Carré. Electrolytes were removed from other portions of the solution by ion exchange and the pectin was determined by electrodeposition and by the calcium pectate method (Table IV).

Analyses by the proposed method gave results from 9 to 13 per cent higher than those obtained by the method of Emmet and Carré.

None of the extracts used in this study contained starch or dextrins, as indicated by the absence of any color formation with iodine solution. It is probable that such interference could be obviated, when necessary, by amylolytic treatment prior to deashing.

Electrodepositions of pectic acid were also made from deashed aliquots of fruit extracts which had been subjected to the same preliminary acetone purification and hydrolysis as used for calcium pectate determination. These results were in good agreement with those obtained by the method of Emmet and Carré (Table V).

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Analysis of Petroleum Oil-Soluble Sodium Sulfonates by Adsorption

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An adsorption method for separating and estimating the components of oil-soap mixtures has been developed, which compares favorably with aqueous alcoholic extraction analyses in accuracy and precision. It is rapid and convenient and entirely free from emulsion difficulties.

IN THE manufacture of medicinal white oil by the treatment of petroleum stocks with fuming sulfuric acid, sulfonic acids are formed, some of which remain dissolved in the oil layer after it is separated from the sulfuric acid sludge. Upon neutralizing the "acid oil" layer with alkali, oil-soluble sodium sulfonates are produced. These "mahogany soaps" are extracted with aqueous alcohol and are further refined to produce commercially important emulsifiers.

Products of this type are marketed principally as a blend consisting of approximately equal amounts of oil and soap, contaminated with small amounts of inorganic matter. To control the ratio of oil to soap in these mixtures, it is necessary to estimate at least one of these components. This may be done by separating the sulfonates from the hydrocarbons by means of some physical process. Distillation is not suitable because of the relatively high boiling points of the hydrocarbons, coupled with the unstable character of the sulfonates at temperatures in the neighborhood of 130° C. Since neither of the components crystallizes readily from their mixtures or from solutions of their blends, crystallization cannot be used to separate them.

Because of the greater solubility of the soaps in a water-alcohol mixed solvent, unsaponifiable matter or oil is usually extracted selectively with petroleum ether, from soap dissolved in 50 per cent aqueous alcohol solution. Most of the oil dissolves in the petroleum ether and the aqueous alcohol retains in solution the major portion of the soap. By a systematic process of multiple extractions, soap and oil mixtures can be separated completely if the proper conditions are chosen. Archibald and Baldeschwieler (*2*) have described a method of analyzing petroleum sulfonates by this type of extraction. Since some of the separations were found to be incomplete, after employing the standardized procedure, the authors have described a method of correcting the oil fraction for the soap contained in it.

The large number of extractions and manipulations ordinarily required by this method are time-consuming, and severe emulsion difficulties are encountered with some types of samples.

Selective adsorption can be used to separate mixtures composed of materials having widely different adsorption characteristics, just as selective solvent action is used to separate materials of different solubilities. The adsorption process is often advantageous because of its convenience, speed, and freedom from emulsion troubles. An adsorbent must be found which will adsorb sodium sulfonates, since oil is not easily adsorbed, and the proper solvent and displacing agent must be chosen. Simple adsorption, like simple extraction, is usually inadequate for sharp separation of two components. Fractionation of some kind must be employed to the best advantage to achieve quantitative results.

This paper presents a new adsorption method which has the advantages of speed, convenience, and freedom from emulsion troubles, and which has been found to be especially useful in this laboratory for plant control work.

PRELIMINARY EXPERIMENTS

Percolation experiments showed that sodium sulfonates could be selectively adsorbed from naphtha solution, and that they could, in turn, be completely displaced from the adsorbent by methanol.

APPARATUS AND MATERIALS

The percolation apparatus, shown in Figure 1, consisted of a 250-ml. separatory funnel, *A*, a 250-ml. extraction flask, *B*, and a percolation tube, *C*. This tube had a 1.5-cm. inside diameter and was 40 cm. long. A delivery tube 5 cm. long and 3 mm. in inside diameter was sealed to the bottom of the percolator. Into it a glass wool plug was packed for the purpose of supporting the adsorbent. Just before starting the percolation, fresh Attapulgus clay was packed into the percolator by tamping successive 5-cm. sections of clay tightly in place with a rod. The clay was 30- to 60-mesh size and had been calcined at 482° C. (900° F.). (Silica gel of 28- to 200-mesh, from the Davison Chemical Corporation, and Fisher adsorption alumina for chromatographic analyses, were tried as sodium sulfonate adsorbents, but were found to be less efficient than Attapulgus clay. The clay was supplied by the Attapulgus Clay Co., 260 South Broad St., Philadelphia, Penna. Approximately 35 grams were used for an analysis.)

Figure 1. Percolation Apparatus

The volatility of the petroleum naphtha solvent used in these experiments was sufficiently above that of the oil in the sample, so that the solvent could be completely separated from the oil by evaporation. Naphtha of 30° to 80° C. (86° to 176° F.) boiling range, distilled from a paraffin-base crude oil, and A.S.T.M. precipitation naphtha (*1*) of 50° to 130° C. (122° to 266° F.) boiling range, were found to be satisfactory.

PROCEDURE

Approximately 2.0 grams of sample are accurately weighed into extraction flask *B*, and dissolved in 25 ml. of petroleum naphtha. The solution is transferred to separatory funnel *A*, which is then stoppered. The stem of the funnel is placed inside the open end of percolator *C*, so that it just touches the clay, as shown in Figure 1. Upon opening the stopcock the solution will percolate down through the clay, and the stoppered separatory funnel will act as an automatic feeding device. The percolate issuing from the bottom of the clay column is caught in the tared extraction flask.

As soon as the last drop of solution has entered the percolator, the stem and inside of the funnel are washed with petroleum naphtha. These washings are charged to the percolator. The top of the percolator is also washed clean of any remnants of sample. Then 100 ml. of naphtha are charged to the separatory funnel and percolated through the clay using the same automatic feeding arrangement described for the solution.

If the total naphtha percolate is clear, it is set aside for evaporation. If it is cloudy, indicating the presence of unadsorbed soap or salts, the combined percolate is run through a second percolator packed with fresh clay. A 100-ml. naphtha wash is used as before. The combined naphtha percolate in the tared extraction flask is very carefully evaporated on a steam bath. Oil is prevented from creeping to the outside wall of the flask by blowing a gentle stream of air into the flask during the evaporation.

While this operation is taking place, 100 ml. of absolute methyl alcohol are percolated through each of the clay columns used to adsorb the soaps. The resulting percolates are collected in a tared extraction flask, and the solvent is evaporated from the soaps in the same manner as was described for the naphtha.

When practically all of the naphtha has been evaporated from the oil fraction on the steam bath, the extraction flask is placed in an oven and kept at 100° to 105° C. for 15 minutes, then cooled and weighed. This process of heating in the oven for 15-minute periods, followed by weighing, is repeated until the loss in weight is less than 0.01 gram. Usually, 15 to 30 minutes of heating are sufficient.

Similar treatment is applied to the soap fraction dissolved in methyl alcohol, except that an oven kept at 120° to 130° C. is used to save time. The weights of oil and soap are reported as per cent of sample taken.

TEST OF THE METHOD

Two tests were applied to this method. For the first test, 40 grams of a refined petroleum sulfonate (sample A, Table II) were separated into an oil and a soap fraction by the method of Archibald and Baldeschwieler (*2*). Settling times of from 8 to 16 hours were permitted for emulsions of naphtha and aqueous isopropyl alcohol to separate sharply into two layers. The oil fraction, however, had an ash content of 0.14 per cent; so it was filtered through Attapulgus clay to produce a colorless oil which contained no ash, and was, therefore, free of soap. A series of mixtures of this oil and the soap fraction was prepared, and then analyzed by the adsorption procedure. Table I contains these analyses and the corresponding known blend values. Sample 5 consisted of the extracted sulfonates themselves, without the addition of any oil.

In the second test, a similar series of known mixtures was prepared by blending oil and soap fractions which had been separated from a group of samples by the adsorption method. The adsorption analyses of these known mixtures are also presented in Table I.

These data indicate that the adsorption procedure gives accurate analyses of oil-soap mixtures of widely varying composition. As shown by the analysis of sample 5, the sodium sulfonates obtained by aqueous isopropyl alcohol extraction contained only approximately 0.3 per cent of oil. The oil fractions obtained in these adsorption analyses were practically colorless and free of soap, as indicated by the absence of ash following their ignition.

PRECISION AND COMPARISON WITH EXTRACTION METHOD

A group of five refined soap samples was analyzed in duplicate to test the precision of the adsorption procedure (Table II). These data, together with the results shown in Table I indicate satisfactory reproducibility for most purposes.

Table I. Analyses of Known Blends of White Oil and Sodium Sulfonates by the Adsorption Procedure

Sample No.	Composition of Sample: According to Blend Soap %	According to Blend Oil %	Found by Analysis Soap %	Found by Analysis Oil %	Deviation Soap %	Deviation Oil %
1	19.6	80.4	20.0	80.0	+0.4	−0.4
2	37.6	62.4	38.0	62.2	+0.4	−0.2
3	58.7	41.3	59.2	40.9	+0.5	−0.4
4	82.5	17.5	82.5	17.6	0.0	+0.1
5	100.0	0.0	100.0	0.3	0.0	+0.3
6	10.1	89.9	10.6	89.7	+0.5	−0.2
7	19.6	80.4	19.8	80.1	+0.2	−0.3
8	35.0	65.0	34.7	65.0	−0.3	0.0
9	50.0	50.0	49.7	49.9	−0.3	−0.1
10	65.0	35.0	64.2	35.1	−0.8	+0.1
11	78.7	21.3	78.2	21.8	−0.5	+0.5
12	89.4	10.6	88.7	11.2	−0.7	+0.6

Samples 1 to 5 were blended from components prepared by aqueous isopropyl alcohol extraction. Samples 6 to 12 were blended from components prepared by Attapulgus clay adsorption.

Table II. Duplicate Adsorption Analyses of Refined Soaps

Sample	Composition by Analysis: Soap %	Composition by Analysis: Oil %	Deviation: Soap %	Deviation: Oil %
A	46.4	53.5	0.1	0.3
	46.5	53.8		
B	50.6	46.2	1.0	0.2
	49.6	46.0		
C	50.4	47.0	0.4	0.3
	50.8	47.3		
D	51.2	49.0	0.1	0.3
	51.3	48.7		
	49.0	50.3	0.8	1.0
	49.8	49.3		

Table III. Comparison of Analyses of Refined Soaps by the Adsorption and Extraction Methods

Sample	Composition by Analysis: Adsorption Method Soap	Oil	Total	Extraction Method Soap	Oil	Total	Deviation Soap	Oil
	%	%	%	%	%	%	%	%
F	56.8	42.7	99.5	56.6	43.1	99.7	+0.2	−0.4
G	60.3	39.1	99.4	59.8	39.6	99.4	+0.5	−0.5
H	55.7	44.4	100.1	54.0	44.8	98.8	+1.7	−0.4
I	56.6	24.8	81.4	58.1	23.4	81.5	−1.5	+1.4
J	67.4	32.8	100.2	66.6	33.4	100.0	+0.8	−0.6

Table IV. Resin-Displacing Power of a Series of Trial Eluants

Trial Eluant	Weight of Material Displaced, *Grams*	Ratio of Material Displaced to Resin on Clay
Acetone	1.490	1.9
Ethyl acetate	0.788	1.0
Diethyl ether	0.786	1.0
Chloroform	0.652	0.8
Ethylene dichloride	0.612	0.8
Nitromethane	0.562	0.7
Carbon tetrachloride	0.136	0.2
Carbon disulfide	0.032	0.0

Five other refined soap samples were analyzed by both the extraction method of Archibald and Baldeschwieler (*2*) and the adsorption procedure and the results are compared in Table III. Samples F and G were from the same manufacturer, but samples H, I, and J came from three different sources. Sample I was found to contain 18.6 per cent of material which was volatile at 130° C.

The agreement between the two methods appears to be satisfactory. They are probably capable of giving equal accuracy and precision on samples of this type.

SOAPS CONTAINING RESINS

Soaps that are produced in the course of medicinal white oil manufacture, as described above, have been found to be practically free of resinous (oxygenated hydrocarbon) components. There are some products on the market, however, which do contain resinous materials. These samples, containing resins in addition to sodium sulfonates and oil, present a more difficult analytical problem for both the extraction and the adsorption methods, than the simpler case of soap and oil mixtures. Whereas the extraction method usually gives somewhat high results for oil on these samples, the adsorption method gives high results for soap, due to the adsorption of resins as well as sodium sulfonates on the clay.

Since there is a difference in molecular structure and polarity between resin and sodium sulfonate molecules, there should also be an appreciable difference in their adsorption affinities. If all the sulfonate molecules in a given sample are more strongly adsorbed by clay than all the resin molecules, the latter can be selectively displaced, by the proper eluant, from a solid on which both components are adsorbed. By a series of trial experiments, ethyl acetate was found to be a good resin eluant. Several publications (*3*, *4*, *5*) have been helpful in choosing eluants.

CHOOSING THE RESIN ELUANT

A sample of soap, prepared by treating a very naphthenic petroleum stock with concentrated sulfuric acid, followed by neutralization with sodium hydroxide, was separated into its chief components: oil, resins, and sulfonates. This was done by extracting the soap from the oily matter (oil-resin mixture) by the aqueous isopropyl alcohol extraction method (*2*), and then separating the resin from the oil by percolating a petroleum naphtha solution of these components through a column of Attapulgus clay. The oil-free resin was recovered from the clay by displacing it with absolute methyl alcohol.

A known test mixture consisting of 34.5 per cent oil, 33.1 per cent resin, and 32.4 per cent soap was prepared and then dissolved in petroleum naphtha, and 25 ml. of this solution, containing 2.359 grams of the test mixture, were percolated through a prepared clay column following the method described in the adsorption procedure. After the clay was washed with 100 ml. of petroleum naphtha, 150 ml. of a trial resin eluant were percolated through the column. This percolate was collected separately, and the weight of material displaced was determined by the method described for soaps. The ratio of this weight to 0.781 gram, the amount of resin in the percolator charge, was calculated.

Table IV contains the data obtained in this manner for a series of organic liquids.

Impure solvents were percolated through an excess of Attapulgus clay to remove small amounts of water and other easily adsorbed impurities.

Acetone displaced practically all of the resin and the sulfonates. Ethyl acetate and diethyl ether displaced a quantity of material practically equal to the weight of resin adsorbed. Since the displaced material contained negligible amounts of ash, it was assumed to be resinous matter. Ethyl acetate and diethyl ether were chosen as being the best resin eluants of the group tested.

MODIFIED ADSORPTION PROCEDURES

To check these conclusions, and to test a modified adsorption procedure for soaps of this type, a group of mixtures containing known amounts of oil, resin, and sulfonates was analyzed. The procedure employed was the same as the one previously described, except that after the 100-ml. petroleum naphtha wash was percolated through the clay, 100 ml. of the resin eluant were percolated in the same manner. The methyl alcohol percolation followed that of the resin eluant. The results of these analyses are shown in Table V.

Table V. Analyses of Known Blends of Oil, Resin, and Sodium Sulfonates by a Modified Adsorption Procedure

Sample No.	Composition of Known Oil	Resin	Soap	Composition by Analysis Oil	Resin	Soap	Resin Eluant
	%	%	%	%	%	%	
1	34.6	33.0	32.4	34.0	26.4	41.3	Chloroform
1	34.6	33.0	32.4	34.8	32.0	33.0	Diethyl ether
1	34.6	33.0	32.4	34.2	60.3	8.8	Acetone
2	55.0	5.0	40.0	57.3	5.7	38.4	Diethyl ether
3	49.8	15.5	34.7	50.5	14.5	34.5	Ethyl acetate
4	59.2	10.9	29.9	60.5	11.1	29.5	Diethyl ether

Table VI. Comparison of Analyses of Soaps Containing Resins by the Extraction and Adsorption Methods

Sample	Extraction Method Soap	Oily matter	Total	Adsorption Method Soap	Oily matter	Total	Deviation Soap	Oily matter
	%	%	%	%	%	%	%	%
1	9.9	89.7	99.6	9.8	90.0	99.8	−0.1	+0.3
2	20.1	80.3	100.4	19.9	79.2	99.1	−0.2	−1.1
3	30.5	70.5	101.0	29.7	69.7	99.4	−0.8	−0.8
4	37.1	63.4	100.5	36.8	63.1	99.9	−0.3	−0.3
5	42.9	57.4	100.3	42.5	57.9	100.4	−0.4	+0.5
6	49.8	51.4	101.2	50.2	49.8	100.0	+0.4	−1.6

Diethyl ether and ethyl acetate were again found to be the best resin eluants, and the modified adsorption procedure using either of these liquids gave analyses of satisfactory accuracy.

In the analysis of petroleum sulfonates containing resins, it is usually sufficient to determine the sodium sulfonate content and the total amount of oily or inactive matter in the sample. The aqueous isopropyl alcohol extraction method (*2*) accomplishes this on samples of this type, but it is time-consuming. The adsorption method can also be applied to obtain only the soap and oily matter in these samples by simply replacing the petroleum naphtha, in the original procedure, with a suitable resin eluant. The first percolate will then contain both the oil and the resins and the methyl alcohol percolate will again contain the sulfonates.

A series of soaps prepared from a very naphthenic stock, as described above, and containing varying amounts of oil and resins, was analyzed by the isopropyl alcohol extraction method (*2*) and

also by the modified adsorption method. Ethyl acetate was used as the solvent for the oily matter, and methyl alcohol as the eluant for the sodium sulfonates. A comparison of the analyses is given in Table VI. Both methods gave essentially the same analyses, but the adsorption procedure was more rapid and convenient.

A known blend containing 49.8 per cent oil, 15.5 per cent resin (65.3 per cent oily matter), and 34.7 per cent sulfonates was analyzed by the ethyl acetate–methyl alcohol adsorption procedure; 66.2 per cent oily matter and 34.0 per cent soap was obtained.

In the case of petroleum sulfonates which are not known to be free of resins, it is therefore advisable to introduce an ethyl acetate percolation at the end of the petroleum naphtha wash, and before the methyl alcohol percolation, to test for the presence of resins. If the ethyl acetate displaces material which leaves practically no ash after ignition, the presence of resins is indicated, and one of the modified adsorption procedures described above should be adopted.

DISCUSSION

The chief advantages of the adsorption procedure are freedom from emulsion difficulties, rapid convenient physical operations, and sharp separations of oil and sodium sulfonate components.

Experience in this laboratory has demonstrated that a given adsorption procedure may fail to give correct analyses on different types of samples. The procedure used will depend upon the adsorption characteristics of the components in the refined oil-soluble sodium soap. Unless the product to be analyzed is known to consist entirely of oil and sodium sulfonates, as is normally the case, the mahogany soaps which are extracted by aqueous alcohol from caustic neutralized acid oil in the manufacture of medicinal white oil, it will be necessary to introduce an ethyl acetate percolation as a test for the presence of resins.

The adsorption procedure can be applied to crude oil-soluble sodium sulfonate products containing appreciable amounts of water and inorganic salts, after first removing these components. This removal can be accomplished very conveniently in the determination of salt and water by the usual methods. For salt, a precipitation-type method such as A.S.T.M. Method D91-40 (*1*) may be used, and for water a modified Dean and Stark method is very convenient.

Adsorption methods should find applications in grease and asphalt analyses, and in the separation of mixtures containing organic salts or acids and hydrocarbons.

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Quantitative Determination of *d*-Galactose by Selective Fermentation

With Special Reference to Plant Mucilages

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A simple method has been developed which permits the determination of small amounts of *d*-galactose in the presence of mannose, glucose, fructose, xylose, arabinose, and glucuronic acid with an accuracy of 92 to 98 per cent. It depends on differential fermentations with two yeasts, *Saccharomyces carlsbergensis* (N.R.R.L. No. 379) which ferments galactose, and *S. bayanus* (N.R.R.L. No. 966) which leaves galactose unfermented. The yeasts have little action on xylose, arabinose, or glucuronic acid, and these compounds do not interfere with the determination. Reducing values of galactose, mannose, and *d*-glucurone were determined using the Munson-Walker method of analysis. The fermentation techniques were successfully applied to the hydrolysis products of lactose and to certain plant mucilages. The possible application of the method to galactose in the presence of galacturonic acid is being studied with a view toward its use in the analysis of other natural products.

RECENTLY, interest in the determination of galactose has been revived because of the newer technological applications of certain mannogalactan mucilages. The estimation of *d*-galactose, which thus assumes a new importance, has always presented difficulties, especially when other carbohydrates were present in quantity. The van der Haar modification of the Kent-Tollens-Creydt method (*4*), which depends on the oxidation of galactose to mucic acid, is not quantitative. Only when rigorous precautions are taken, and when relatively large amounts of galactose are present, does the procedure give fairly accurate results (*14*).

Ever since the earlier investigations of Kluyver (*7*), the quantitative estimation of *d*-galactose by fermentation with certain yeasts has interested chemists and microbiologists. Kluyver found that galactose was fermented by two varieties of *Saccharomyces cerevisiae* and by a "milk sugar yeast". He also showed that *Schizosaccharomyces pombe*, *Torula monosa*, and *T. dattila* did not ferment galactose. On the basis of these differences, he proposed a proximate method for the microbiological determination of galactose in the presence of other sugars. Among those who used (and modified) Kluyver's procedures were Schmidt, Trefz, and Schnegg (*11*), Hopkins, Peterson, and Fred (*6*), Sherrard and Blanco (*13*), Kurth and Ritter (*9*), Kurth (*8*), Scott and West (*12*), Harding, Nicholson, and Grant (*5*), and, very recently, Menzinsky (*10*). The last author showed that the strain of *Saccharomyces cerevisiae* which he used in galactose fermentation required preconditioning by culturing the yeast on a

galactose-containing medium. Without such pretreatment the yeast was unable to utilize galactose.

Besides the yeasts mentioned above, the following are known to ferment galactose: *Saccharomyces pastorianus* (*3*), *S. marxianus* (*3*, *6*), and *S. fragilis* (*10*). Other yeasts known to ferment the common hexoses other than galactose are *S. productivus* (*3*), *S. apiculatus* (*3*), and "*Honey B*" *yeast* (*6*). These lists are not exhaustive.

Kluyver used the evolution of carbon dioxide as a measure of fermentable sugars. Later investigators (*6*, *8*) showed that a quantitative measure of the reducing value simplified the analysis of fermented sugar solutions.

Notwithstanding the extensive work on the selective fermentation of galactose, the results of relatively few experiments with pure sugar mixtures have been published and no attempt has been made to determine whether the methods could be applied in the presence of uronic acids. The limitations and general applicability of the fermentation methods are, therefore, indeterminate. The present study shows the usefulness of the microbiological method.

EXPERIMENTAL

The objects of the present investigation were to examine, more critically than heretofore, the application of differential fermentations to known sugar mixtures, noting their limitations, and to develop a proximate biochemical method for the determination of galactose in the hydrolyzates obtained from mucilages and hemicelluloses.

Several strains of *S. cerevisiae*, cultured in the laboratories of The Institute of Paper Chemistry, had been shown to ferment galactose quantitatively in 1937 by Kurth (*8*). In 1942, however, qualitative experiments with these same strains showed that none fermented 1 per cent galactose solutions completely within 190 hours. It is evident that strains of *S. cerevisiae* may lose their potency as galactose fermenters with time, unless special precautions are taken to recondition them.

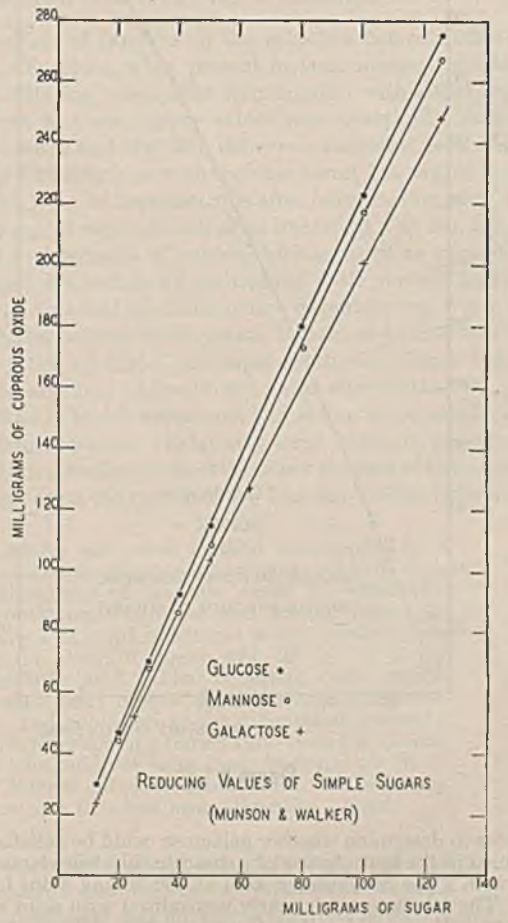

Figure 1

Torula dattila appeared at first to give fairly promising results as a nonfermenter of galactose. About 92 to 95 per cent of the galactose (in a 1 per cent solution), when treated with *Torula dattila*, could be recovered after 2 days at 30° C., but only about 80 per cent remained after a 5-day fermentation period. Whenever the concentrations of galactose dropped to 0.1 per cent, the sugar was rapidly destroyed.

The use of these organisms was discontinued in favor of two interesting yeasts obtained from L. J. Wickerham, Northern Regional Research Laboratory, Peoria, Ill. These were *Saccharomyces carlsbergensis* var. *mandshuricus*, N.R.R.L. No. 379 (originally obtained from Charles N. Frey of the Fleischmann Laboratories in 1940), a highly fermentative strain acting on dextrose, galactose, and some of the common disaccharides, and *S. bayanus*, N.R.R.L. No. 966 (also originally obtained from Frey in 1940), which was known to ferment dextrose, sucrose, and maltose, but which, qualitatively at least, had no effect on galactose. Neither yeast had (during the past three years) been kept on galactose media.

These organisms proved entirely satisfactory. Neither had more than a slight effect on arabinose, xylose, and glucuronic acid. No. 379 fermented *d*-glucose, mannose, fructose, and galactose almost quantitatively within 48 hours. No. 966 fermented the first three readily within the same time period and showed no action whatsoever on galactose. These differences led to the development of a satisfactory proximate method based on differential fermentations, in which the Munson-Walker reduction method (*2*) was used without modification. In aliquot portions, taken from fermentation mixtures, 20 to 125 mg. of galactose could be determined with an accuracy of 92 to 98 per cent, even when other sugars were present originally in great excess.

The yeasts were maintained in good condition by monthly transfers on glucose agar (Bacto-Dextrose agar, dehydrated). They have shown no decrease in potency over a period of 8 months. Agar slant cultures, 2 to 7 days old, were used for the preparation of the suspensions required in inoculating the sugar solutions. The following procedure was the same for either yeast.

About 2 ml. of sterile water were pipetted into the tube containing the agar slant culture, and the surface growth was removed gently by means of the pipet, which also served to stir the suspension briskly. For each bottle slant of glucose agar required, 0.5 ml. of the suspension was removed and spread over the surface by tilting the bottle back and forth. Eight-ounce, narrow-mouth, square, flint glass bottles with molded screw caps were used as containers for sterile water, yeast suspensions, and bottle slants. Thereupon, the slants were incubated for about 48 hours at 30° C. One bottle slant easily furnished enough inoculum for four subsequent sugar analyses, because a dense growth of yeast cells coated the agar surface at the end of the 48-hour period.

About 10 ml. of sterile water were added to the bottle slant, and the bottle was tilted back and forth to loosen the growth. About 5 ml. of the dense yeast suspension were pipetted into a sterile dilution bottle, and 20 to 30 ml. of sterile water were added. The exact amount of water depended upon the turbidity shown by a Cenco-Sheard-Sanford photelometer. Suspensions whose readings fell within the range of 10 to 16 on this instrument, when distilled water gave a reading of 90, subsequently fermented sugar mixtures satisfactorily. If the initial photelometer reading fell below 10, more water was added and thoroughly mixed with the suspension. Whenever the photelometer reading exceeded 16, more yeast suspension was added. In practice it was found better to work with too dense than with too light a suspension. The density range listed above was shown by plate counts to approximate 35,000,000 cells per ml. This density also corresponded roughly to that of barium sulfate suspension prepared by mixing 5 ml. of a stock solution of 10 grams of barium chloride dihydrate per liter of water with 55 ml. of 1 per cent sulfuric acid and allowing the mixture to stand at least a week in a sealed con-

Table I. Action of Yeasts on Pentoses after a 6-Day Incubation Period

Pentoses Present in Aliquot	Treatment	Cu_2O Obtained in Munson-Walker Determinations
Mg.		*Mg.*
12.5 (xylose)	Control	26.3
12.5 (xylose)	Organism 379	20.8
12.5 (xylose)	Organism 966	22.3
12.5 (arabinose)	Control	28.6
12.5 (arabinose)	Organism 379	26.7
12.5 (arabinose)	Organism 966	26.7

tainer. It was found expedient to use 25 ml. of sugar solutions in the fermentations and to carry out all experiments in 125-ml. Erlenmeyer flasks. The total reducing sugar in such solutions never exceeded 2 per cent, and the galactose concentration was ordinarily kept within the range of 40 to 250 mg. per 25 ml. To the sugar solution were added 15 ml. of a filtered yeast extract. [Red Star starch-free yeast cake was mixed with sufficient distilled water to yield a 10 per cent suspension. This was heated for 1 hour in an Arnold sterilizer at about 100° C. and subsequently for 20 minutes at 15 pounds' pressure (at 121° C.). The cooled suspension was filtered several times through fluted filter paper using Cellite to clarify the solution. Ordinarily the yeast extract formed a slightly turbid solution. This was dispensed in 80-ml. portions into Erlenmeyer flasks, which were plugged with cotton and heated at 15 pounds' pressure for 20 minutes. The cooled flasks of sterile yeast extract can be stored for months without change in a refrigerator.]

The sugar and yeast extract mixtures, which showed a pH of about 5 to 6 (alkacid paper), were then sterilized at 15 pounds' pressure for 15 minutes, cooled to about 30° C., inoculated under aseptic conditions with 10 ml. of the appropriate yeast suspension, and incubated at 30° C. for a minimum of 48 hours.

The fermentations were always run concomitantly in pairs, under identical conditions, one flask being inoculated with organism 966 and the other with organism 379. Three or four times during the incubation period, the flask was rotated gently to bring the bottom yeasts into intimate contact with the sugar solution. At the end of the fermentation period, each solution was diluted to 100 ml. with distilled water, thoroughly mixed, and filtered through two No. 50 Whatman filter papers. Twenty-five to 50-ml. aliquot portions of the clear, pale yellow filtrates were taken for analysis by the usual Munson-Walker technique. (Care must be taken to digest the asbestos used in Gooch crucibles thoroughly with hot Fehling solution, and with concentrated nitric acid. To prevent later clogging of Gooch crucibles, such treatments should be continued until asbestos filter pads permit the rapid filtration of hot Fehling solution containing the filtered yeast extract referred to above.)

The weight of cuprous oxide resulting from the fermentation with organism 379 was subtracted from that obtained by the use of organism 966. The galactose equivalent was calculated by the use of the galactose–cuprous oxide graph (Figure 1) drawn from data obtained experimentally with pure galactose. (The mannose values given in Figure 1 were obtained from pure *d*-mannose; the glucose values were taken from Munson and Walker's tables.)

The effects of *S. carlsbergensis* and *S. bayanus* on small amounts of xylose and arabinose were shown to be relatively unimportant, even after an incubation period of 144 hours instead of the usual 48-hour period. This is indicated in Table I (and Table II).

Inasmuch as the net reducing values in all differential galactose determinations were obtained by subtracting the weight of cuprous oxide found after fermentation with organism 379 from that found after a fermentation with organism 966, the over-all error resulting from the presence of pentoses is negligible. Furthermore, the authors' quantitative fermentation periods seldom exceeded 2 days, which presumably would result in a lessened action on the pentoses.

Because glucuronic acid may be a minor component of hemicellulose hydrolysis, the effect of *d*-glucurone in the galactose analysis was determined. Figure 2 shows the reducing values in milligrams of cuprous oxide plotted against the weights of glucurone (melting point 174–5° C.) taken for analysis. Over a fairly wide range, this curve is practically coincident with that of glucose. The action of organisms 379 and 966 on glucurone was found to be very slight and their over-all effect in differential fermentations of galactose was almost negligible. This is indicated in the last row of Table II.

Orientating experiments were also carried out with purified galacturonic acid, which remains virtually unattacked by either organism, even when only small amounts of the acid are present in the usual fermentation mixture. The reducing value (Munson-Walker) of 12.5 mg. of galacturonic acid was shown to be 23.0 mg. of cuprous oxide; when neutralized, sterilized, inoculated, and incubated in the usual manner, 20.7 mg. and 20.2 mg. of cuprous oxide were obtained with organisms 379 and 966. Here again the "error" is cancelled.

The value and limitations of the selective differential fermentations in the determination of galactose are clearly shown in Table II. The first and last columns of this table should be compared. Invariably, in the higher concentrations, galactose shows a slight but persistent residual reduction after fermentation with organism 379. Whether this is due to very small amounts of unfermented galactose or (what is more probable) to the slight reducing power of the products of the fermentation is not known. The error is never very appreciable, but it accounts for the fact that galactose recoveries are usually somewhat low. Organism 966 is without effect on galactose.

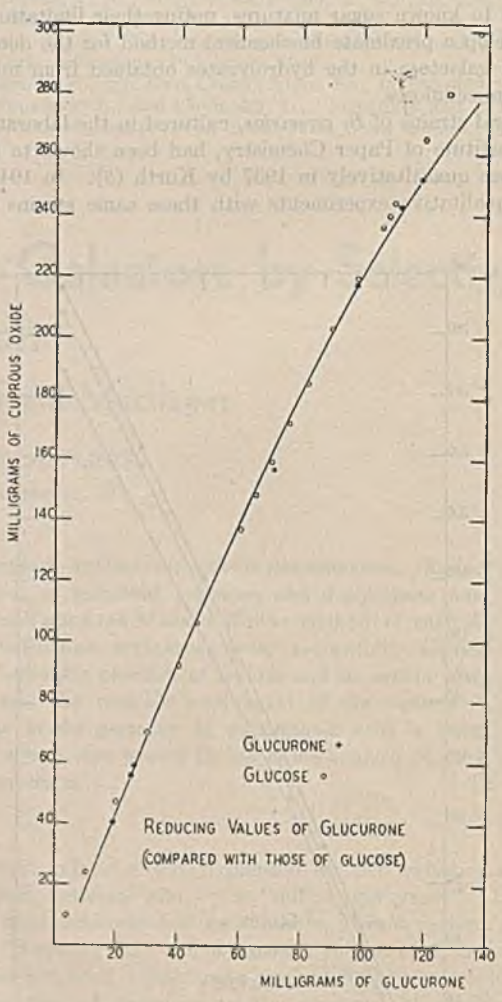

Figure 2

In order to determine whether galactose could be satisfactorily determined in the hydrolyzate of a disaccharide, pure lactose was heated with 2 per cent sulfuric acid at the boiling point for 2.75 hours. The solutions were nearly neutralized with solid sodium carbonate (pH about 5, alkacid test paper). Aliquot portions representing, in each case, 125 mg. of lactose (hydrate) were sub-

Table II. Galactose Determination Alone and in Mixtures of Pure Sugars

1 Galactose Taken	2 Other Components	3 Cu_2O Obtained after Fermentation with No. 966	4[a] Cu_2O Obtained after Fermentation with No. 379	5 Net Weight of Cu_2O, 3–4	6 Galactose Recovered (Calcd. from 5, by Use of Figure 1)
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
125	None	247.0	2.6	244.4	123.5
125	None	250.1	5.7	244.4	123.5
125	None	247.3	4.5	242.8	123.0
150 (Fermented 72 hrs.)	None	292.6	6.0	286.6	146.5
None	Mannose 125	None	None	None	None
None	Mannose 125	Negligible <1	Negligible <1	None	None
62.5	Mannose 62.5	126.6	1.7	124.9	61.5
45	Mannose 80	91.2	1.6	89.6	42.5
25	Mannose 100	49.5	0.5	49	23.5
20	Mannose 230	37.1	None	37.1	18.5
40	Mannose 40 Glucose 40 Xylose 5	88	8.8 (due to xylose)	79.2	37.5
40	Mannose 20 Glucose 20 Xylose 45	173	92.0[b] (due to xylose)	81.0	38.5
25	Fructose 25.0 Mannose 62.5	51.2	1.6	49.6	23.8
30	Fructose 30.0 Mannose 75.5	60.5	None	60.5	29.0
37.5	Glucurone 12.6	101.55	25.75[c] (due to glucurone)	75.8	36.0

[a] Unless otherwise stated, figure in column 4 represents error due to incomplete fermentation of galactose or to presence of small amounts of reducing substances among galactose fermentation products.
[b] Recovered (using Allihn's factor), 40.7 mg. of xylose.
[c] Recovered, 13 mg. of glucurone.

jected to differential fermentation. The galactose values found were 62.5 and 62.5 mg. (=126.5 and 126.6 mg. of cuprous oxide); the calculated value is 62.5 mg. of galactose.

The effect of fructose on the galactose determination is negligible. Fructose, when present in large amount, shows a persistent reducing value after fermentation with either organism. Inasmuch as these copper values were practically identical for both No. 379 and No. 966, the errors cancelled each other. In a set of fermentations with fructose alone, 250 mg. of this sugar yielded 7.1 mg. of cuprous oxide after fermentation with No. 966, and 6.9 mg. of cuprous oxide after treatment with No. 379.

Acid hydrolyzates of polysaccharides (such as gums or hemicelluloses) are ordinarily neutralized with purified barium carbonate. To avoid the introduction of barium ion, which may or may not be deleterious to yeasts, sodium carbonate was used in lowering the acidity of the sugar solutions. These were never rendered alkaline. Alkacid test paper showed that the pH was about 5 to 6, which experience had shown to be satisfactory for yeast fermentations. Relatively large amounts of sodium sulfate had practically no effect on either the rate of fermentation of the sugar or on the results of the Munson-Walker determination.

The above analytical method was applied to a series of mannogalactan mucilages isolated from the endosperms of various seeds by extraction with hot water, filtration, and precipitation with ethanol. The dried mucilages were hydrolyzed by boiling for about 6 hours with 25 ml. of 2 per cent sulfuric acid. The solutions were cooled, brought to a pH of 5 to 6 with solid sodium carbonate, and subjected to the differential fermentation without removing the solution from the 125-ml. flask. The analyses were made as usual on 25- or 50-ml. filtered aliquots taken from the fermentation mixtures that had been diluted to 100 ml.

The sugar yields found in such hydrolyses should be considered minimal values. Significant amounts of mannose were lost on hydrolysis, but galactose appeared to be largely unaffected. This was shown by control experiments with pure sugar solutions that had been treated with 2 per cent sulfuric acid for 6 hours. One hundred milligrams of mannose yielded 217 mg. of cuprous oxide before and 210 mg. of cuprous oxide after acid treatment. The mannose reversion products, however, had no effect on the galactose determination. The acid-treated mannose fermented completely without reducing value. Galactose appeared to be unaffected by the action of 2 per cent sulfuric acid; 100 mg. of galactose yielded 200.5 mg. of cuprous oxide before and 201.0 mg. of cuprous oxide after acid treatment.

Table III gives the yields of galactose (calculated as galactan on the oven-dry, ash-free basis) of several endosperm mucilages, some of which have distinct technological interest.

DISCUSSION

Assuming a modicum of microbiological control, the relative simplicity of the differential fermentation method for galactose has obvious advantages over the older mucic acid procedure. It requires less material for analysis, less attention on the part of the analyst, and is less subject to fluctuations with slight variations in technique. It also appears to be more accurate.

S. carlsbergensis (organism N.R.R.L. No. 379) requires no reconditioning to galactose, such as that required by certain strains of *S. cerevisiae*. Organism N.R.R.L. No. 379 ferments galactose readily, despite the fact that this sugar has not been used as a nutrient in its culture. In the authors' brief experience, *S. carlsbergensis* does not lose its potency on transfer. It was as active in fermenting galactose after 8 months of culture as it was on receipt from Peoria. *S. bayanus*, although a powerful hexose fermenter, leaves galactose practically untouched. This difference in behavior should make for an ideal combination of microorganisms. On the other hand, the galactose fermentation by No. 379 leads to products which have a slight reducing value (as shown in Table II). Although very slight, the error thus caused must be taken into account. The reduction is also subject to minor fluctuations. In general, it leads to galactose values that are slightly low.

The usefulness of the method in its application to certain gums and mucilages is manifest. In the case of the endosperm mucilages, independent determinations of mannose (*1*), coupled with the figures given in Table III, account for 94 to 97 per cent of the total hydrolyzates. The possible application of the galactose method to the hydrolysis products of pectins, in which galacturonic acid residues predominate, is under investigation. How-

Table III. Galactan Content of Plant Mucilages

Mucilage	Galactan, %	Mucilage	Galactan, %
Guar 1 (*Cyamopsis tetragonalobus*)	37.8, 38.2[a] 37.4, 37.6[a]	Arabogalactan (from W. Larchwood)	79.4, 79.5[a] 80.1
Guar 2	33.8, 34.4[a]	Palo verde (*Cercidium torreyanum*)	21.3, 21.9[a]
Locust bean (*Ceratonia siliqua*)	20.0 19.9	Tara (*Caesalpinia spinosa*)	26.2, 26.4[a]
Honey locust (*Gleditsia triacanthos*)	26.0 25.9		
Flame tree (*Delonix regia*)	18.2, 18.9[a]	Huizache (*Caesalpinia cacaloca*)	27.7, 27.3[a]
Kentucky coffee bean (*Gymnocladus dioica*)	26.2, 26.8[a] 25.4, 25.6[a]	*Sophora japonica*	15.6

[a] Duplicate determinations on aliquots from same fermentation are given on same line.

ever, a few preliminary experiments indicate that galacturonic acid is affected but little by either organism.

ACKNOWLEDGMENT

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Use of the Discriminant Function in the Comparison of Proximate Coal Analyses

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Comparing a number of analyses of a material from one source with analyses of similar material from another source has heretofore presented a very real problem. The present paper applies statistical methods to the comparison of proximate analyses and B.t.u. per pound of coal from two mines. This statistical analysis by random sampling shows the probability that two coals come from the same source. The discriminant function developed by Fisher is used. By means of this function a comparison may be obtained between analytical data based on material from one source and data on similar material from a different source. In addition, the order of significance of the several analytical constituents in a single series of samples may be determined. The method is capable of general application.

FISHER (*6*), in 1936, developed the discriminant function for the comparison of multiple measurements obtained in taxonomic problems. Since then three papers (*3, 4, 5*) have appeared which make use of Fisher's technique. The present paper is concerned with the application of the discriminant function to the differentiation of two series of proximate coal analyses and the B.t.u. per pound of coal. Each series of analytical data is from a different mine.

Each series consists of 100 samples of coal. The proximate analysis, covering the volatile matter, fixed carbon, per cent of ash, as well as the B.t.u. per pound of coal, is used in making the comparison of the coal from these mines. The samples were taken from cars of coal sent to this college over a period of several years, and the analyses are reported on samples dried at 105° C. The analytical data, while accurate, indicate that the methods of sampling may be questioned. The value of the present approach is, however, not in the data reported but in the application of statistical procedures to comparison of similar chemical data. The discriminant function enables one to obtain a numerical comparison of the coals by the use of two linear compounds or equations in which the effects, in the present instance, of all four of these measurements are combined. Further, the application of this function to these analytical data permits a test for significance between the constituents of which the compounds are formed.

The compound for the first mine is:

$$X = b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4$$

where x_1, x_2, x_3, and x_4 represent, respectively, B.t.u., per cent of volatile material, per cent of fixed carbon, and per cent of ash, and b_1, b_2, b_3, and b_4 are constants to be found. The variables x_1, x_2, x_3, and x_4 may be correlated. The compound for the second mine is

$$X' = b_1x_1' + b_2x_2' + b_3x_3' + b_4x_4'$$

where x_1', x_2', x_3', and x_4' represent, respectively, the above similar measurements; the coefficients are the same as in compound X. The difference between the means of the above two compounds made up of the four measurements is

$$D = b_1d_1 + b_2d_2 + b_3d_3 + b_4d_4 \qquad (1)$$

where $d_1 = \overline{x_1} - \overline{x_1'}$, $d_2 = \overline{x_2} - \overline{x_2'}$, $d_3 = \overline{x_3} - \overline{x_3'}$, and $d_4 = \overline{x_4} - \overline{x_4'}$, and $\overline{x_1}$, $\overline{x_2}$, $\overline{x_3}$, and $\overline{x_4}$ represent the arithmetic means of the respective measurements made of the coal from the first mine and $\overline{x_1'}$, $\overline{x_2'}$, $\overline{x_3'}$, and $\overline{x_4'}$ represent the means of similar measurements made on the coal from the second mine.

Table I. Measurements of B.t.u., Per Cent of Volatile Material, Per Cent of Fixed Carbon, and Per Cent of Ash for Mines A and B

Mine A				Mine B			
B.t.u., x_1	Volatile matter, x_2	Fixed carbon, x_3	Per cent ash, x_4	B.t.u., x_1'	Volatile matter, x_2'	Fixed carbon, x_3'	Per cent ash, x_4'
13,000	25.7	64.3	7.9	13,600	33.0	59.6	6.5
13,700	25.0	64.2	8.4	14,300	36.9	56.3	6.2
12,800	23.0	66.2	10.0	13,000	35.5	50.9	12.9
12,300	22.8	59.9	12.9	14,000	34.9	58.7	5.8
14,100	33.5	60.2	5.9	13,700	30.1	59.3	10.0
...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...
13,900	27.3	59.4	8.3	13,300	35.2	54.8	9.2
Av. 13,110	28.33	61.16	8.71	13,596	34.00	56.28	8.65

Table I contains a few measurements from mines A and B, taken at random from the sets of observations. The means of the 100 measurements on each of four properties are given in the last line of that table.

Let

$$S = b_1^2 s_{11} + b_2^2 s_{22} + b_3^2 s_{33} + b_4^2 s_{44} + 2b_1 b_2 s_{12} + 2b_1 b_3 s_{13} + 2b_1 b_4 s_{14} + 2b_2 b_3 s_{23} + 2b_2 b_4 s_{24} + 2b_3 b_4 s_{34}$$

where

$$s_{ij} = \sum_{k=1}^{100} (x_{i,k} - \overline{x_i})(x_{j,k} - \overline{x_j}) + \sum_{k=1}^{100} (x'_{i,k} - \overline{x'_i})(x'_{j,k} - \overline{x'_j})$$

where k is the variable of summation. The value of s_{ij} when $i = 1$ and $j = 1$ is

$$s_{11} = \sum_{k=1}^{100} (x_{1,k} - \overline{x_1})^2 + \sum_{k=1}^{100} (x'_{1,k} - \overline{x'_1})^2$$

In our case this quantity is (using values in Table I) found as follows:

$$s_{11} = (13{,}000 - 13{,}110)^2 + (13{,}700 - 13{,}110)^2 + \ldots + (13{,}900 - 13{,}110)^2 + (13{,}600 - 13{,}596)^2 + (14{,}300 - 13{,}596)^2 + \ldots + (13{,}800 - 13{,}596)^2 = 20{,}110{,}000 + 17{,}718{,}400 = 37{,}828{,}400$$

The value of s_{ij} where $i = 2$ and $j = 3$ is

$$s_{23} = \sum_{k=1}^{100} (x_{2,k} - \overline{x_2})(x_{3,k} - \overline{x_3}) + \sum_{k=1}^{100} (x'_{2,k} - \overline{x'_2})(x'_{3,k} - \overline{x'_3})$$

By using the values in Table I this is

$$s_{23} = (25.7 - 28.33)(64.3 - 61.16) + (25.0 - 28.33)(64.2 - 61.16) + \ldots + (27.3 - 28.33)(59.4 - 61.16) + (33.0 - 34.00)(59.6 - 56.28) + (36.9 - 34.00)(56.3 - 56.28) + \ldots + (35.2 - 34.00)(54.8 - 56.28) = -645.82 - 373.49 = -1{,}019.31$$

The quantity S is equal to the sum of squares within compounds. By maximizing the quantity D^2/S the following equations arise. These equations are actually proportionalities, but for the purpose of evaluating constants b_1, b_2, b_3, and b_4 they may be used.

$$\begin{aligned} s_{11}b_1 + s_{12}b_2 + s_{13}b_3 + s_{14}b_4 &= d_1 \\ s_{12}b_1 + s_{22}b_2 + s_{23}b_3 + s_{24}b_4 &= d_2 \\ s_{13}b_1 + s_{23}b_2 + s_{33}b_3 + s_{34}b_4 &= d_3 \\ s_{14}b_1 + s_{24}b_2 + s_{34}b_3 + s_{44}b_4 &= d_4 \end{aligned}$$

From these equations the values of the b's, of the coefficients in compound X, can be found. The solution of these equations gives, of all linear compounds in x_1, x_2, x_3, and x_4, the one compound, X, which most clearly discriminates one mine from the other. These equations are:

$$37{,}828{,}400.00b_1 + 123{,}906.00b_2 + 20{,}685.20b_3 - 140{,}927.00b_4 = 486.00$$

$$123{,}906.00b_1 + 1{,}614.87b_2 - 1{,}019.31b_3 - 571.21b_4 = 5.67$$

$$20{,}685.00b_1 - 1{,}019.31b_2 + 1{,}849.62b_3 - 849.97b_4 = -4.88$$

$$-140{,}927.00b_1 - 571.21b_2 - 849.97b_3 + 1{,}519.42b_4 = -0.06$$

There are several ways of solving these equations. One way (2), using the computing machines, is as follows: Dividing each equation by the absolute value of the coefficient of b_1 in it gives:

$$(A)\quad b_1 + 0.003275b_2 + 0.000547b_3 - 0.003725b_4 = 0.000013$$

$$(B)\quad b_1 + 0.013033b_2 - 0.008226b_3 - 0.004610b_4 = 0.000046$$

$$(C)\quad b_1 - 0.049277b_2 + 0.089418b_3 - 0.041091b_4 = -0.000236$$

$$(D)\quad -b_1 - 0.004053b_2 - 0.006031b_3 + 0.010782b_4 = 0.000000$$

$$(A) - (B)\quad -0.009758b_2 + 0.008773b_3 + 0.000885b_4 = -0.000033$$

$$(B) - (C)\quad 0.062310b_2 - 0.097644b_3 + 0.036481b_4 = 0.000282$$

$$(C) + (D)\quad -0.053330b_2 + 0.083387b_3 - 0.030309b_4 = -0.000236$$

Dividing each equation by the absolute value of the coefficient of b_2 in it gives:

$$(E)\quad -b_2 + 0.899057b_3 + 0.090695b_4 = -0.003382$$

$$(F)\quad b_2 - 1.567068b_3 + 0.585476b_4 = 0.004526$$

$$(G)\quad -b_2 + 1.563604b_3 - 0.568329b_4 = -0.004425$$

$$(E) + (F)\quad -0.668011b_3 + 0.676171b_4 = 0.001143$$

$$(F) + (G)\quad -0.003464b_3 + 0.01714b_4 = 0.000101$$

Dividing each equation by the absolute value of the coefficient of b_3 in it gives:

$$(H)\quad -b_3 + 1.012215b_4 = 0.001711$$

$$(I)\quad -b_3 + 4.950058b_4 = 0.029157$$

$$(H) - (I)\quad -3.937843b_4 = -0.027446;\ b_4 = 0.006970$$

From (H) $b_3 = 0.005344$
From (I) $b_3 = 0.005345$; $b_3 = 0.005345$ (average)

From (E) $b_2 = 0.008820$
From (F) $b_2 = 0.008821$
From (G) $b_2 = 0.008821$; $b_2 = 0.008821$ (average)

From (A) $b_1 = 0.000007$
From (B) $b_1 = 0.000007$
From (C) $b_1 = 0.000007$
From (D) $b_1 = 0.000007$; $b_1 = 0.000007$ (average)

Therefore

$$b_1 = 0.000007,\ b_2 = 0.008821,\ b_3 = 0.005345,\ b_4 = 0.006970$$

This method of solving simultaneous equations is easy to follow and easy to explain to the average computer.

The linear compound which enables one to detect the greatest difference between the mines in relation to these four measurements is

$$X = 0.000007x_1 + 0.008821x_2 + 0.005345x_3 + 0.006970x_4$$

The mean compound pertaining to mine A is

$$\overline{X} = 0.000007\overline{x_1} + 0.008821\overline{x_2} + 0.005345\overline{x_3} + 0.006970\overline{x_4} = 0.000007\ (13{,}100) + 0.008821\ (28.33) + 0.005345\ (61.16) + 0.006970\ (8.71)$$

or

$$\overline{X} = 0.7293$$

The mean compound pertaining to mine B is

$$\overline{X}' = 0.000007\ (13{,}596) + 0.008821\ (34.00) + 0.005345\ (56.28) + 0.006970\ (8.65) = 0.7562$$

The difference D between these two means is $0.7562 - 0.7293 = 0.0269$. This can be found directly from Equation 1 as follows:

$$D = 0.000007\ (486) + 0.008821\ (5.67) + 0.005345\ (-4.88) + 0.006970\ (-0.06)$$

or

$$D = 0.003402 + 0.050015 - 0.026083 - 0.000418 = 0.0269 \quad (2)$$

as before.

The question arises as to whether or not the means of these compounds are statistically significantly different. Table II contains an analysis of variance of these compounds and enables

one to test for a significant difference between these two means. The two asterisks in the last column indicate that these compounds are highly significantly different. This means that the probability of getting by chance such a large value of D is less than 0.01. This is found from a table of F values (*1*, *7*). The odds in favor of getting by chance such a large difference between the compounds pertaining to the two mines are less than 1 to 99. This indicates that the coal from mine A is definitely different from the coal from mine B, as far as these four measurements are concerned.

By examining the four terms in Equation 2 one can determine which characteristic of the coal (B.t.u., per cent of volatile material, per cent of fixed carbon, or per cent of ash) is most important for differentiating the two coals, which is of next importance, etc. The second term, 0.050015, in this equation is greater than the absolute value of each of the other terms; hence the per cent of volatile material is the most important factor, in this case, for determining whether or not the coal from one mine is different from the coal from the other mine. The absolute value of the third term in this equation, 0.026083, is second in size; hence the per cent of fixed carbon is next in importance for revealing a difference between the coal from the two mines. The factor B.t.u. is third in importance for differentiating these two coals. Per cent of ash is of the least importance for these mines. The order of importance of these factors may change for other coals. Table III gives the ranks of importance of these characteristics when various combinations are used to calculate the compounds.

Table III gives the ranks of the characteristics of the coals for various combinations of the sets of measurements. In column 2 it is seen that per cent of fixed carbon is the most important characteristic for differentiating one mine from the other as far as per cent of volatile material, per cent of fixed carbon, and per cent of ash are concerned.

Table II. Analysis of Variance of the Compounds

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	199	....	....
Between compound means	4	$50D^2 = 0.0362$	0.00905**
Within	195	$D = 0.0269$	0.00014

Table III. Rank of Characteristics for Various Compounds Pertaining to Coals A and B

	Compound Composed of				
	B.t.u. Per cent of volatile matter Per cent of fixed carbon	Per cent of volatile matter Per cent of fixed carbon Per cent of ash	B.t.u. Per cent of volatile matter	Per cent of carbon Per cent of ash	Per cent of volatile matter Per cent of fixed carbon
B.t.u.	3	..	2	..	..
Volatile matter	1	2	1	..	2
Fixed carbon	2	1	..	1	1
Per cent ash	..	3	..	2	..

Table IV contains the means of measurements pertaining to B.t.u., volatile material, fixed carbon, and per cent of ash for two other mines, C and D. The discriminant function pertaining to these mines for the value D is

$$D = 0.000020\,(495) - 0.006360\,(5.10) - 0.001559\,(-2.63) - 0.000468\,(-2.16)$$

or

$$D = 0.00990 - 0.00350 + 0.00410 + 0.00101$$

$$D = 0.01151$$

An analysis of variance table (not given) shows that the two coals are significantly different. The characteristic B.t.u. is most important, per cent of fixed carbon is second, per cent of volatile material is third, and per cent of ash is last in importance in testing whether or not the mines differ as far as a compound of these four sets of measurements is concerned.

Table IV. Arithmetic Averages of B.t.u., Per Cent of Volatile Material, Per Cent of Fixed Carbon, and Per Cent of Ash Measurements from Mines C and D

Mines	B.t.u.	Per Cent of Volatile Matter	Per Cent of Fixed Carbon	Per Cent of Ash
C	13,700	35.09	57.18	6.56
D	13,205	29.99	59.81	8.72
Difference	495	5.10	−2.63	−2.16

Table V. Averages of B.t.u., Per Cent of Volatile Material, Per Cent of Fixed Carbon, and Per Cent of Ash for Mines E and F

Mines	B.t.u.	Per Cent of Volatile Matter	Per Cent of Fixed Carbon	Per Cent of Ash
E	14,012	34.15	60.31	4.21
F	12,134	35.78	48.14	13.25
Difference	1,878	−1.63	12.17	−9.04

Table V contains averages of measurements pertaining to B.t.u., volatile material, fixed carbon, and per cent of ash for mines E and F.

The difference between the means of the two discriminant functions pertaining to the mines is

$$D = 0.000083\,(1878) - 0.001356\,(-1.63) + 0.006124\,(12.17) + 0.009580\,(-9.04) = 0.155874 + 0.002210 + 0.074529 - 0.086603 = 0.146010$$

In this case B.t.u. is first, per cent of ash is second, per cent of fixed carbon is third, and per cent of volatile material is fourth in importance for differentiating between the coal from these mines.

DISCUSSION

The discriminant function enables one to test for a statistical difference between two linear compounds made up of several variables or measurements. It has many advantages because it furnishes one measurement pertaining to a combination of several measurements.

In the present instance the statistical analysis of the data shows a difference between the coal from the two mines. It may be useful in the future to compare analytical data from other mines. Such an effort would involve the selection of a standard coal, and it seems apparent that other analytical data, such as per cent of sulfur, per cent of moisture, and the fusing temperature of the ash, should be included in the more accurate statistical comparison.

CONCLUSION

Fisher's discriminant function has been applied to the differentiation of analytical data obtained from one hundred coal samples taken from each of two mines. The intercomparison of the relative significance of the elements of the analytical data relating to coal has been accomplished. The probability that two coals come from the same source by random sampling is given.

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Colorimetric Analysis of Xanthone Spray Residues

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A colorimetric method for the determination of xanthone spray residues consists in adding a measured quantity of toluene to a sample of apples or apple plugs in a glass jar and shaking for 5 minutes. The resulting solution of xanthone and apple waxes is filtered and an aliquot of the filtrate taken for analysis. The xanthone is reduced to xanthydrol by refluxing with sodium amalgam in toluene and methanol. After removal of the methanol by a water extraction, an aliquot of the supernatant toluene solution is swirled in concentrated hydrochloric acid, effecting an equilibrium transfer of the xanthydrol to the acid layer to give a yellow color, which is measured photometrically.

XANTHONE has been used experimentally as an insecticide against codling moth larvae and other insects. The purpose of this study was to develop a satisfactory method for determining xanthone residues on sprayed apples, and the following colorimetric procedure is recommended. It is based on a procedure used by the Laurel Hill Laboratory of the General Chemical Company for determining xanthone spray residues. The writers are indebted to several of the staff of this laboratory for constructive criticism and advice in the preparation of this paper.

ANALYTICAL PROCEDURE

REAGENTS. Toluene. Toluene may contain an impurity, probably a thiophene derivative, which upon shaking with concentrated hydrochloric acid yields a slight yellow color in the acid layer. This impurity can be removed by adding 50 ml. of concentrated sulfuric acid per liter of toluene, allowing to stand over 24 hours, separating the layers, and distilling the toluene. The first milky portion of the distillate is discarded. Toluene from all operations may be recovered and reused if treated in this manner. White crystals of *p*-toluenesulfonic acid may appear in the toluene layer during the sulfuric acid treatment, but they do not distill and do not interfere. C.P. toluene usually does not contain this impurity.

Methanol (absolute).

Sodium amalgam. Cautiously melt 9 grams of sodium in 20 ml. of toluene in a round-bottomed flask and add 750 grams of mercury, drop by drop at first and more rapidly after a few milliliters have been added. Most of the toluene will volatilize, but some should be kept over the amalgam when it is transferred to an airtight bottle.

Hydrochloric acid (C.P. concentrated).

PREPARATION OF STANDARDS. Carefully weigh 50.0 mg. of pure xanthone, transfer to a 250-ml. volumetric flask, and make to volume with toluene. Keep tightly stoppered to prevent loss of toluene. One milliliter of this solution contains 200 micrograms of xanthone. If pure xanthone is not available for standards, it can be prepared by distilling a crude xanthone product and recrystallizing the distilled material several times from dioxane or other suitable solvent to a constant melting point of 174° C. Measure 2 ml. of the standard solution and sufficient toluene to make a total of 20 ml. into a 125-ml. flask fitted with a ground-glass joint. Add 10 ml. of methanol and 0.5 to 1.0 ml. of sodium amalgam, connect with a water-cooled condenser, and reflux for 30 minutes. Before removing the flask from the condenser, cool to prevent loss of toluene. Add 20 ml. of water and shake vigorously to remove the methanol from the toluene. Pour into a tall tube, such as a 50-ml. Nessler tube, retaining the amalgam in the flask. Allow the layers to separate, and pipet 5 ml. of the toluene layer (containing the xanthydrol) into a 100- to 150-ml. flask that contains exactly 10 ml. of concentrated hydrochloric acid. Develop the color by swirling the mixture gently for approximately 1 minute. Pour into a test tube or cell and measure the color of the acid layer in a photometer. A glass color filter having maximum transmission at 424 millimicrons gave satisfactory results in a Type F Aminco photometer.

Repeat for 5- and 10-ml. aliquots of standard xanthone solution. The 5/20 aliquots of toluene used for color development represent 100, 250, and 500 micrograms of xanthone. Prepare a standard graph by plotting the quantities of xanthone in the standard solution against the logarithms of the corresponding photometer readings. The light transmission of 148 micrograms of xanthone read under the conditions above in a 2.5-cm. (1-inch) cell is 50 per cent.

ANALYSIS OF SAMPLES. A statistical analysis has shown that 20 to 25 apples, taken from different parts of a tree, constitute a satisfactory sample for residue determination. Place the apples in a tared glass jar, weigh, and calculate the surface area from the weight, by the use of a previously established relationship. Add from 100 to 250 ml. of toluene, depending on the quantity of xanthone and the size of the apples, and shake for 5 minutes in a machine by the process described by Fahey *et al.* (*1*). Filter a portion of the solution, and use an aliquot not to exceed 10 ml. of the filtrate for the analysis as described under the procedure for standards. Read the amount of xanthone per aliquot from the standard graph.

Apple plugs may be treated in a similar manner. If they are used, a smaller volume of toluene can be used for stripping.

DISCUSSION

SOLVENTS FOR XANTHONE. In selecting a solvent for the removal of xanthone residues, consideration was given to solubility, efficacy of wax removal from apples, and the extraction of interfering substances. Acetone, alcohols, benzene, and petroleum ether were not satisfactory because of poor solvent power for xanthone and too great solvent power for interfering substances.

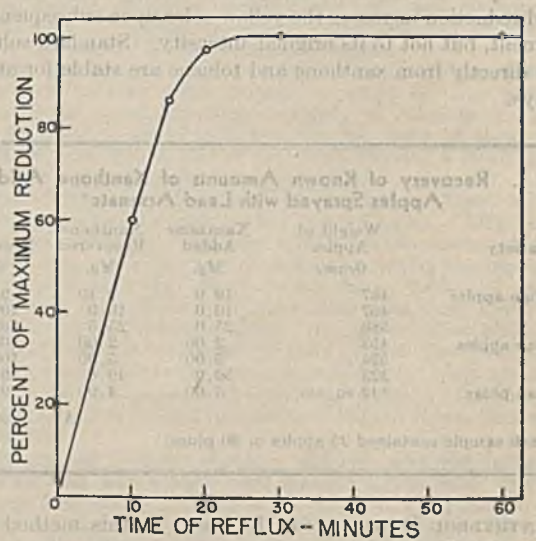

Figure 1. Rate of Xanthone Reduction to Xanthydrol in Toluene-Methanol Mixture

The solubility of xanthone in toluene is 1.43 gram per 100 ml. at 30° C., which is far in excess of any concentration normally encountered in residue analysis. Since the apple wax appears to be completely dissolved, any xanthone that may be covered with wax is also obtained. The amount of interference introduced by shaking even mature waxy apples with toluene for 30 minutes is negligible. On the other hand, 5 minutes' shaking is sufficient for complete removal of xanthone residues. Toluene was therefore selected as the most desirable solvent for this method.

REDUCTION OF XANTHONE. It is best to use an aliquot of the residue solution containing from 0.4 to 2.0 mg. of xanthone for reduction. If the aliquot contains larger quantities, up to 50 mg., reduction will be complete, but further aliquoting and dilu-

tion after removal of the methanol will be necessary. The term "complete reduction" as used here means that, under the conditions of the method, a more intense yellow color cannot be obtained with a given amount of xanthone even if the time of reduction is doubled. Reduction of xanthone in a mixture of 20 ml. of toluene and 10 ml. of methanol reaches a maximum, under the reflux condition of the method, in 25 minutes (Figure 1), as judged by color development.

Color Development. After reduction the addition of water followed by vigorous shaking removes the methanol from the toluene layer, which returns to its original volume of 20 ml. When the mixture is transferred to a container for the separation of the two layers, it is convenient to withdraw the amalgam for subsequent recovery. The 5-ml. aliquot of the toluene layer needed for color development can be taken before all the toluene has separated; it is not even necessary to filter to remove slight water turbidity. If smaller aliquots are used, sufficient pure toluene to make 5 ml. must be added before treatment with acid.

When xanthydrol is treated with hydrochloric acid, chlorine is substituted for the hydroxyl group. The resulting compound is colorless in dilute acid, but in the presence of concentrated hydrochloric acid an intense yellow color is produced. The intensity of yellow color is proportional to the quantity of xanthone used in the determination. If the toluene is removed from the acid layer to prevent a shift in the distribution ratio, the solution can be diluted with concentrated hydrochloric acid and the color still conforms to Beer's law.

No decomposition of xanthydrol has been observed in toluene up to 12 hours after reduction, but low recoveries were obtained on some samples when the toluene layer was allowed to stand for a longer time. It is possible that the observed decomposition is due partly to oxidation of xanthydrol to xanthone, because a renewed reduction increases the yellow color upon subsequent acid treatment, but not to its original intensity. Standard solutions made directly from xanthone and toluene are stable for at least 60 days.

Table I. Recovery of Known Amounts of Xanthone Added to Apples Sprayed with Lead Arsenate[a]

Variety	Weight of Apples	Xanthone Added	Xanthone Recovered	Recovery
	Grams	*Mg.*	*Mg.*	%
Jonathan apples	467	10.0	9.40	94.
	467	10.0	10.0	100.
	586	25.0	25.5	102.
Winesap apples	453	2.00	2.00	100.
	524	5.00	5.00	100.
	323	50.0	49.6	99.
Winesap plugs	142 sq. cm.	5.00	4.90	98.
			Av.	99.0

[a] Each sample contained 25 apples or 80 plugs.

Xanthydrol Distribution Ratios. In this method there are two distributions of xanthydrol, between toluene and water-methanol solution and between toluene and hydrochloric acid. Both distribution ratios have been found to be constant for any total amount of xanthydrol up to 50 mg. for the former and 0.6 mg. for the latter, when the volumes are kept as specified in the method. The distribution ratio between toluene and hydrochloric acid was not studied beyond 0.6 mg., because the intensity of color at the corresponding concentration was more than sufficient for the method. Two per cent of the xanthydrol remains in the water-methanol solution, and 77 per cent of the total is transferred to the hydrochloric acid. The concentration of the hydrochloric acid is not too critical, since experience has shown no color differences in the range of 34 to 36.5 per cent acid, and whereas the use of lower strength acid will lead to lesser color intensities, constant results will be obtained if the standards are treated with the same acid. If conditions require the use of volumes other than those specified, a new standardization curve must be established. Since distribution ratios are involved in the method, it is imperative that all volumes be measured accurately.

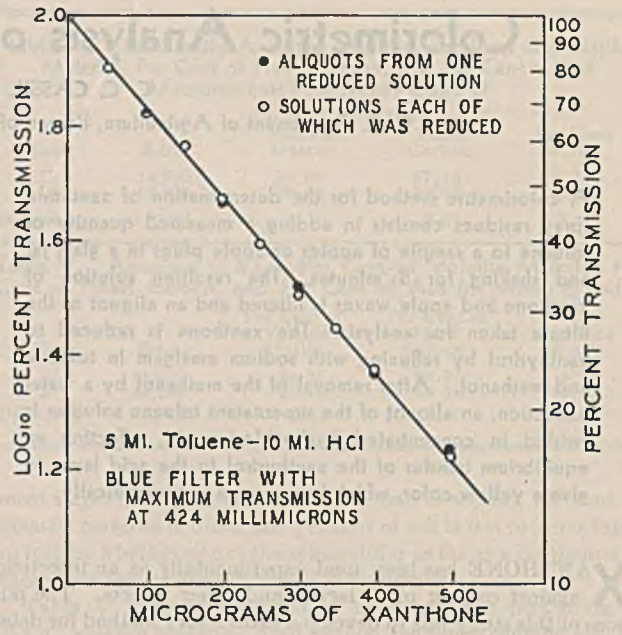

Figure 2. Representative Standard Xanthone Graph

When an aliquot is taken from the supernatant toluene layer, there is some tendency for redistribution of the xanthydrol upon standing 2 hours or longer. This effect is the more pronounced the larger the concentration of xanthydrol. No explanation can be given for this change in distribution, but it is constant for any constant volume ratio. If the procedure is followed as described, good results are obtainable. If the toluene solution is to be retained for checking color development on the same day, it should be separated from the water-methanol solution before an aliquot is removed.

The distribution of xanthydrol between toluene and hydrochloric acid rapidly comes to equilibrium, but as a check one should make duplicate determinations at this point in the procedure. After color development no change in intensity occurs, even after 24 hours, if precautions are taken with respect to apple-wax concentration in the toluene as described in the next section.

Interferences. Apples sprayed with lead arsenate alone and stripped for 5 minutes in toluene gave a photometer reading corresponding to that given with 0.1 microgram of xanthone per square centimeter. Similar samples stripped for 30 minutes did not show an interference greater than 0.15 microgram per square centimeter. When this type of interference was tested on Delicious, Rome, and Winesap varieties, no significant differences were found. The blank on apple plugs is also negligible. Xanthone deposits of 22 micrograms per square centimeter dropped to 1.6 micrograms after weathering for 4 weeks; therefore, if any decomposition products are formed, they either do not remain on the apple or do not cause any detectable interference with the method.

Apple wax does not interfere in the reduction of xanthone. It also causes no interference with the color developed in hydrochloric acid when the aliquot of strip solution is 10 ml. or less. It is possible to use a 15-ml. aliquot or even 20-ml. with early-season apples, but if turbidity occurs in the acid phase, the wax concentration in toluene must be reduced in some way. The yellow color developed in hydrochloric acid is stable for 24 hours or more, but should be read within an hour, since turbidity may develop on long standing. This effect is most marked when the

toluene is in contact with the acid layer overnight and is probably due to temperature changes.

Ground-glass joints are preferred for the reflux reduction. Rubber stoppers may be used if they are boiled in normal alkali for 15 minutes, then in normal sulfuric acid for 15 minutes longer, and finally rinsed well with distilled water.

Accuracy and Precision. One standard graph obtained in this investigation is given in Figure 2. The absorption cells used were standard test tubes (2-cm. inside diameter). The line was fitted by the method of least squares. The standard error of estimate for the points on this line is ±6.7 micrograms. Aliquots can be so chosen as to permit readings on 200 to 500 micrograms of xanthone, thus allowing reduction of the percentage standard error to 3.3 or less. More accurate and precise results can be obtained if the colored solutions are read in cells with flat optical windows, since the test tubes used in this work vary about 0.5 per cent in light transmission.

Recovery experiments were run by adding known amounts of xanthone to apples that had been sprayed with lead arsenate, and analyzing by the described procedure (Table I). The average recovery from seven samples was 99.0 per cent, which is not statistically different from complete recovery.

The completeness of removal of xanthone from Winesap and Rome apples (collected 6 weeks before harvest) was also studied by submitting samples to a second stripping with toluene. The amount of xanthone removed by the second treatment was calculated after allowing for the amount of toluene left on the apples from the first stripping. Six samples, having from 6 to 13 mg. of xanthone per sample, treated in this manner, showed a removal of 99 per cent or better with the first 5-minute stripping treatment.

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PHENOL STUDIES

Qualitative Tests for Phenol and o-, m-, and p-Cresol

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Qualitative tests for phenol employing ferric chloride, hypochlorite, or the reagents of Melzer, Millon, Liebermann, Guareschi, and Cotton have been modified to permit differentiation between phenol and o-, m-, and p-cresol.

THE manufacture and use of phenol and cresol on a large scale have furnished an incentive for investigating the toxicity and metabolism of these compounds, and these investigations, in turn, have called for a review of analytical methods useful in their detection and estimation. A review of quantitative methods for the estimation of phenol in biological material, including a spectrophotometric procedure for the quantitative estimation of free, conjugated, and total phenol in tissues and fluids, has been published (*2, 3*). In this paper qualitative tests for *o-*, *m-*, and *p*-cresol which are modifications of well known qualitative tests for phenol are described. The hypochlorite test for *o*-cresol offered here has apparently not been recorded before.

A single test or a combination of several of these color tests can be used very effectively for the identification of phenol or *o-*, *m-*, or *p*-cresol, if the unknown solution contains only one of these compounds. If the unknown contains two or more of these substances, positive identification of each compound is not always possible; *m*-cresol, when present in mixtures in a low concentration, is particularly apt to escape recognition.

In analyzing for these compounds, even though each substance gives very similar color reactions in high and in low concentrations, it is best to prepare and test dilutions that approach the ranges of sensitivity. These concentrations will furnish in addition some rough idea of the quantities present.

The phenol used was Merck's reagent quality, and the *o-*, *m-*, and *p*-cresol, obtained from the Eastman Kodak Company, was believed to be from 96 to 98 per cent pure. The melting points of these cresols are 30–31°, 10–11°, and 32–34° C., respectively.

QUALITATIVE TESTS

Detection of Phenol and Derivatives Containing Phenol-Hydroxy Grouping. The sensitivity of Millon's test (*7*) depends to some extent upon the quantity of mercury used and the manner of preparing the reagent. For these studies the latter was prepared by dissolving 497 grams of mercury in 700 ml. of nitric acid (sp. gr. 1.42) and diluting this solution with 2 volumes of water. One milliliter of the test solution is added to 2 ml. of Millon's reagent.

Phenol and *o*-cresol when present to about 1.0 mg. per ml., and *m-* and *p*-cresol when present to about 0.5 mg. per ml., produce a red color almost immediately at room temperature. When reduced to about 0.05 mg. per ml., each of these compounds produces in the cold or on careful heating a straw-yellow color which is destroyed by further heating.

Modification of Melzer's Benzaldehyde Test (*6*) for Detection of Phenol and *o-*, *m-*, and *p*-Cresol. Mix 1 ml. of the aqueous solution to be tested with 2 ml. of concentrated sulfuric acid (sp. gr. 1.84), and after cooling under the tap add 2 drops of benzaldehyde. Heat over a flame, cool, and add 10 ml. of water and 20 ml. of 40 per cent potassium hydroxide. The sensitivity of this test is about 1 mg. per ml. of solution.

Modification of Guareschi's Test (*4*) for Distinguishing Phenol and *p*-Cresol from *o-* and *m*-Cresol. Add about 0.5 gram of solid potassium hydroxide and 3 drops of water to 3 ml. of a chloroform extract containing phenol or cresol, then warm and observe.

Straw-colored (yellow) globules will rise and the potassium hydroxide and water layer will assume a yellowish tinge on warming if phenol or *p*-cresol is present. In the presence of *o-* or *m*-cresol, the potassium hydroxide and the water layer will assume a pinkish or rose-red color. The sensitivity for each of these compounds is about 4 mg. in 3 ml. of extract.

Ferric Chloride Test (*8*) for Detection of *o-* and *p-*

Table I. Color Changes

Phenol	*o*-Cresol	*m*-Cresol	*p*-Cresol
Changes observed in 5 minutes after addition of sulfuric acid and benzaldehyde			
Cloudy olive	Cloudy orange	Cloudy yellow	Milky white
After heating			
Cloudy reddish-brown	Cloudy brownish-red	Cloudy yellowish-brown	Cloudy brownish-green
After cooling and addition of water and potassium hydroxide			
Blue or violet solution and precipitate		Colorless or faintly tan colored solution and precipitate	

CRESOL. Add 2 drops of a freshly prepared 10 per cent aqueous solution of ferric chloride to 5 ml. of the test solution.

Phenol and *m*-cresol produce clear bluish-purple colors, *o*-cresol produces in about 10 minutes a slightly cloudy urine yellow or brown solution, while *p*-cresol produces a cloudy blue solution. The sensitivity for each compound is about 10 mg. in 5 ml.

MODIFICATION OF LIEBERMANN'S TEST (*5*) FOR DETECTION OF *p*-CRESOL. To 3 ml. of the unknown aqueous solution add slowly and with shaking 1 ml. of the reagent (6 per cent solution of sodium nitrite in concentrated sulfuric acid). A cloudy orange solution develops in about 5 minutes if *p*-cresol is present. Phenol and *m*-cresol yield clear, and *o*-cresol very slightly cloudy brown or yellowish-brown solutions. The sensitivity of this test is about 5 mg. in 3 ml.

MODIFICATION OF COTTON'S TEST (*1*) FOR DETECTION OF *p*-CRESOL. To 3 ml. of an aqueous solution, add 1 ml. of concentrated ammonium hydroxide (sp. gr. 0.901) and 4 drops of the freshly prepared reagent (10 ml. of concentrated hydrochloric acid and 0.5 gram of potassium chlorate added to 40 ml. of water).

Phenol and *o*- and *m*-cresol produce in 5 to 10 minutes clear light blue colors; *p*-cresol produces a clear light straw-yellow color. The sensitivity is about 10 mg. in 3 ml. of solution.

HYPOCHLORITE TEST FOR DETECTION OF *o*-CRESOL. To 5 ml. of the test solution, add one drop of sodium hypochlorite solution. In the presence of *o*-cresol the solution will immediately turn a cloudy yellowish-white; in the presence of phenol, and *m*- and *p*-cresol, it will remain clear and colorless. The sensitivity is about 4 mg. in 5 ml. of solution. (Excess of hypochlorite must be avoided because it may produce faint cloudiness with *p*-cresol.)

DISCUSSION

It is reasonable to assume that all reagents discussed in this paper will also react with some compounds other than phenol, or *o*-, *m*-, or *p*-cresol. Therefore one must make certain that the test solution is comparatively free from compounds related to phenol or cresol. This may require preliminary precipitation, extraction, or distillation procedures.

Millon's test, even though it makes specific differentiation between phenol and the three cresols difficult, is of value because of its simplicity, as a preliminary test. This should be followed by the modified tests of Melzer and Guareschi, which will identify the compound. The conclusions drawn from these latter two tests may be checked by the hypochlorite and ferric chloride tests or by the modified procedures of Liebermann and of Cotton.

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Stability of Standard Solutions of Copper Perchlorate and Potassium Iodate

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WHENEVER sodium thiosulfate is used in titration work of high accuracy frequent restandardization is necessary. In order to avoid the troublesome and wasteful necessity of preparing for each standardization a fresh solution of a primary standard [iodine, potassium iodate (*3*), copper perchlorate (*2*)], a standard in the form of a solution of a primary substance of dependable stability is desirable. The work reported here deals with the possibility of using potassium iodate or copper perchlorate (*2*) for such a purpose.

In considering the stability of such solutions it is necessary to distinguish between changes due to chemical instability, presumably resulting in decrease of active concentration, and changes due to evaporation from the container, which will cause increases of concentration. In the study of Berman (*1*), for instance, on the stability of potassium iodate, it is impossible to distinguish the role of these two factors. However, his data suggest that evaporation has been an appreciable factor in his results, and that, in some cases, an apparent stability has resulted from the opposing effects of evaporation and decomposition.

In a recent paper (*4*) on the stability of sodium thiosulfate solutions no account was taken of the possible effect of evaporation. A graphical study of the data on stability shows that in the first 60 days there is approximately a 0.3 per cent increase in normality. After that the values drop. Again two antagonistic tendencies, evaporation and decomposition, tend to produce a false picture of the stability of thiosulfate solutions.

The evaporation factor can be eliminated if, at the beginning of the experiment, samples of the solution to be examined are pipetted into separate flasks, and some of these are titrated at once, while others are titrated after a suitable lapse of time. The extent of evaporation, on the other hand, can be measured by suitable weighings of the vessels containing stock solutions. If the solution is chemically stable, it is then possible to calculate the theoretical normality at any time from the initial normality and the loss of weight that has occurred.

APPARATUS AND REAGENTS

A 50-cc. buret and one 10-cc. pipet were carefully calibrated and were used throughout the experiments. Details of the preparation and use of the copper perchlorate and potassium iodate are given in (*2*) and (*3*), respectively.

EXPERIMENTAL

All titrations were carried out in duplicate. The amount of active substance present in solutions when they were fresh, and after they had stood for various lengths of time, was always determined by titration with thiosulfate (0.025*N*), newly standardized against two freshly prepared cupric perchlorate solutions (*2*).

Table I. Stability of 0.1*N* Copper Perchlorate and Potassium Iodate Solutions

Solution	Days Standing	Normality Initial	Normality Final[a]	Conditions
$Cu(ClO_4)_2$	565	0.1007	0.1002	Thymol, glass stoppers
	565	0.1007	0.1004	Glass stoppers
	289	0.0993	0.0992	Cork stoppers, spores on corks
	454	0.0993	0.0993	
KIO_3	565	0.1027	0.1021	Glass stoppers
	565	0.1027	0.1013	Thymol[b], glass stoppers
	565	0.1027	0.0983	
	289	0.1007	0.1002	Cork stoppers, spores on corks
	454	0.1007	0.1004	

[a] Final normality calculated on basis of its initial volume.
[b] Only one sample; others are average value found for two samples.

Table II. Stability of Solutions in Glass-Stoppered Bottles

Days	Loss of Weight per 100 Days		Normality		Container	Bottle Capacity	Approximate Volume of Solution
	% of weight of solution present	Grams	Calculated	Found		Cc.	Cc.
					Copper Perchlorate		
0	...	...	...	0.1005	Glass-stoppered Pyrex about 12 years old	250	155
0–85	0.740	1.218	0.1011	0.1013			
85–290	0.679	0.898	0.1026	0.1025			
290–355	0.445	0.534	0.1033	0.1034			
0	...	...	...	0.1003	Glass-stoppered, flint	500	165
0–85	0.675	1.112	0.1009	0.1009			
85–290	0.488	0.671	0.1019	0.1023			
290–455	0.506	0.576	0.1028	0.1034			
0	...	...	...	0.1007	Rubber stopper, Pyrex	250	120
0–185	0.018	0.021	0.1007	0.1007			
0	...	...	...	0.0993	Glass stopper, Pyrex, sealed with paraffin	250	145
0–373	0.033	0.048	0.0994	0.0994			
373–1159	0.083	0.075	0.1001	0.0997			
					Potassium Iodate		
0	...	...	...	0.1007	Glass stopper, flint glass; mold after several months	500	265
0–85	0.053	0.141	0.1007	0.1007			
85–453	0.095	0.156	0.1011	0.1010			
0	...	...	...	0.1028	Brown, glass stopper, paraffined	500	195
0–368	0.032	0.064	0.1029	0.1026			
368–768	0.021	0.036	0.1030	0.1021			
0	...	...	...	0.1001	Standard interchangeable glass stopper, Pyrex	250	160
0–157	0.051	0.083	0.1002	0.1000			
157–557	0.035	0.050	0.1003	0.0997			
0	...	...	...	0.1027	Glass stopper and ground-joint cap ("ether bottle")	500	185
0–238	0.021	0.040	0.1028	0.1027			
0	...	...	...	0.1027	Rubber stopper	250	165
0–165	0.022	0.036	0.1028	0.1013			
0	...	...	...	0.1028	Flint, glass stopper, turbid, deposit of inorganic material on walls	1 liter	85
0–855	0.130	0.113	0.1040	0.1037			

In the experiments summarized in Table I, 10-cc. samples of freshly prepared cupric perchlorate and potassium iodate solutions were pipetted into 125-cc. Erlenmeyer flasks. Some solutions were titrated at once, while other flasks were closed with either glass stoppers or fresh cork stoppers and protected against dust by paper caps. These flasks were stored in the laboratory in a cabinet that was opened frequently and thus provided no protection against possible contamination from the laboratory atmosphere. The temperature was 22° to 35° C. The last of these solutions were titrated after 19 months. As a possible prevention of mold growth, about 10 mg. of thymol were added to some of the flasks.

Table III. Effect of Storage under Conditions of Minimum Evaporation

Solution	Days	Loss of Weight per 100 Days		Normality		Conditions
		% of weight of solution present	Grams	Initial	Final	
$Cu(ClO_4)_2$	368	0.018	0.019	0.1022	0.1018	Glass stopper, Pyrex
KIO_3	392	0.0018	0.0018	0.1002	0.0999	Glass stopper, flint

Table I shows the results. It appears that copper perchlorate solutions possess a high degree of stability, while potassium iodate solutions show a pronounced tendency to become weaker. In either case thymol has a harmful effect, presumably because it is oxidized, while in the copper perchlorate solutions, even the presence of black, sporelike spots on the cork stoppers was not associated with any loss of titer.

In the experiments summarized in Table II, samples of freshly prepared copper perchlorate and potassium iodate solutions were titrated with thiosulfate. The remaining portions of the solutions were transferred to clean, dry, tared, glass-stoppered reagent bottles and weighed with an accuracy of ±5 mg. After standing for varying periods under the conditions described above, the bottles were carefully dusted and about half an hour later weighed. A pair of 10-cc. samples were withdrawn and titrated as above. The bottles were then reweighed and the above procedure was repeated at intervals. From the data given, one can readily calculate the initial weight of the solutions. In some cases, portions of the solutions were removed for other purposes. The bottles were weighed before and after such removals and due corrections were applied in the calculations.

In order to keep evaporation at a minimum the following procedure was tried. A tared 250-cc. glass-stoppered bottle containing about 100 cc. of copper perchlorate solution was weighed. The bottle, placed in a dry beaker, was stored in a desiccator over a portion of the same solution. One year later the bottle was taken from the desiccator, wiped, and carefully weighed as before. Duplicate 10-cc. samples were then titrated. A potassium iodate solution was treated in the same manner. A comparison of the results obtained (Table III) with those shown in Table II shows that by the use of good glass-stoppered or rubber-stoppered bottles nearly the same results can be attained as by the desiccator method.

CONCLUSIONS

Solutions in glass-stoppered bottles may lose weight by evaporation. This loss in weight has a tendency to give some types of solutions an appearance of stability. Results of experiments indicate that solutions of copper perchlorate are more stable than those of potassium iodate. For use as permanent standards copper perchlorate solutions should be stored in good glass-stoppered or rubber-stoppered bottles (evaporation losses should be determined by weighing, and normalities should be corrected accordingly). Iodate solutions must be stored in glass-stoppered bottles. Thymol should not be used as a preservative for either copper perchlorate or potassium iodate solutions.

ACKNOWLEDGMENT

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Semiautomatic Pressure Control in Low-Pressure, Low-Temperature Laboratory Fractionation

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THE necessity of using laboratory personnel manpower to the best advantage and the desire continually to improve the quality of fractional analysis have led to the installation of automatic (*2*) and semiautomatic control (*1*, *2*, *4*) on fractionating columns in many laboratories. In the past, even semiautomatic pressure control (*2*, *3*) has entailed the use of an elaborate device installed at no small expense. The pressure control devices described by Bosschart (*1*) and Podbielniak (*2*) employ compressed air for dispensing liquid nitrogen to the column head by means of electrically operated valves controlled by electrical contacts in the column manometer.

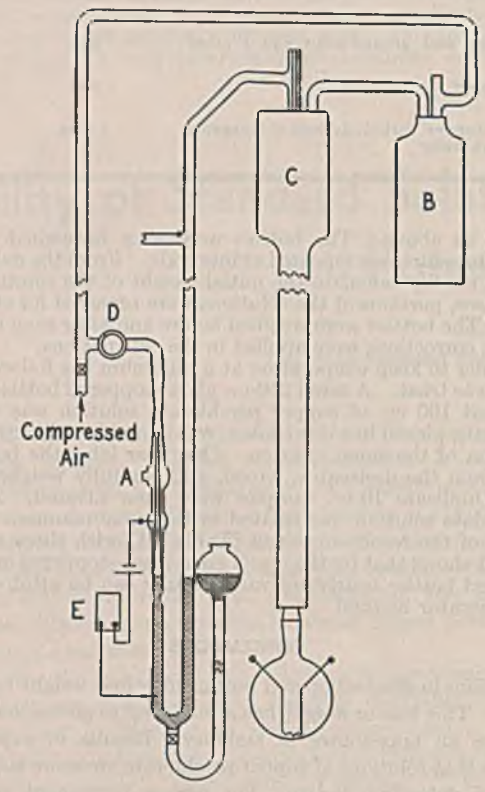

Figure 1. Semiautomatic Pressure Control Device

A. Throttling valve
B. Liquid nitrogen dispenser
C. Column condenser
D. Manual control
E. Electrical buzzer

This paper describes a control device developed in this laboratory from materials usually available, which provides a simplified means of semiautomatic control for low-temperature, low-pressure laboratory fractionating equipment, without sacrificing excellence of control or ease of operation. Its outstanding attributes are simplicity of construction and low cost. The device may be used with good results on either the standard low-temperature laboratory fractionating column or the Podbielniak Heli-Grid type (*3*); and if these two types of columns are connected to the same manifold, the control may be shifted from one column to the other simply by moving the nitrogen flask. No other changes in the pressure control mechanism are necessary.

The principal features of the control device are shown in Figure 1, in relation to the fractionating column. Three essential parts of the mechanism are shown in detail: the throttling valve in Figure 2, and the nitrogen dispenser and nitrogen expander in Figure 3.

THROTTLING VALVE

The throttling valve (Figure 2) is an extension of the outer arm of the column manometer, and is actuated by mercury which rises out of the manometer and into the valve, causing the needle, *a*, to float on the meniscus of the mercury column. The ball on top of the needle will move in the throat, *b*, of the valve with rise and fall of mercury. This motion will restrict the freedom of a stream of air which normally exhausts through the vent, *c*, on the valve and must, as a result of the throttling effect of the needle, create sufficient pressure within the nitrogen-dispensing bottle to cause a discharge of liquid nitrogen. The needle will seek an equilibrium position in the throat of the valve, effecting a small but constant discharge of nitrogen just sufficient to control the pressure in the column and maintain it with very slight fluctuation. The amount of mercury in the manometer may be varied to produce any pressure plateau desired from 0 to 760 mm.

The dimensions of the throttling valve are somewhat arbitrary; but 7-mm. glass tubing for the column manometer and lower section of the control valve in which the needle rides was found to give satisfactory results. The following dimensions are suggested: length of throat section, 7.5 cm.; diameter at narrowest section of throat, 2 to 2.5 mm.; length of needle, 9 cm. The throat section can be drawn down to proper size from 10- or 11-mm. glass tubing; the main precautions are to keep the glass circular and as thick as possible. After a satisfactory throat section has been drawn, it should be sealed to tubing of the proper size (7 mm.). The ball on top of the needle should just pass through the narrowest section of the throat without sticking, and the bottom of the needle must ride freely in the tube on the mercury meniscus. The most satisfactory method for connecting the throttling valve to the column manometer is shown in Figure 4. The three-way stopcock should be at least 25 mm. below the 760-mm. mark on the meter stick to allow a length of manometer tube to take care of pressure build-up when recharging the nitrogen bottle.

The needle in the throttling valve described above never seats, but allows exhaust air to flow through at all times. The movement of the needle in the tapered section varies the size of the orifice formed by the throttling valve, which acts to control the flow of exhaust air, thereby producing control of pressure within the nitrogen-dispensing system. The position of the ball on the needle in the lower tapered section of the throat of the valve restricts the flow of air through the valve just enough to create sufficient pressure within the nitrogen-dispensing system to force the amount of liquid nitrogen out of the liquid nitrogen flask needed to control column pressures and to maintain liquid reflux in the column. As the nitrogen level in the flask decreases, more

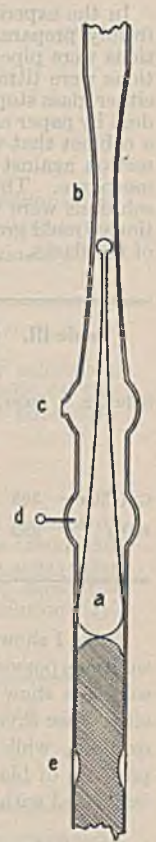

Figure 2. Throttling Valve

a. Floating needle
b. Throat section
c. Air exhaust
d. Tungsten electrical contact
e. Constriction in tubing

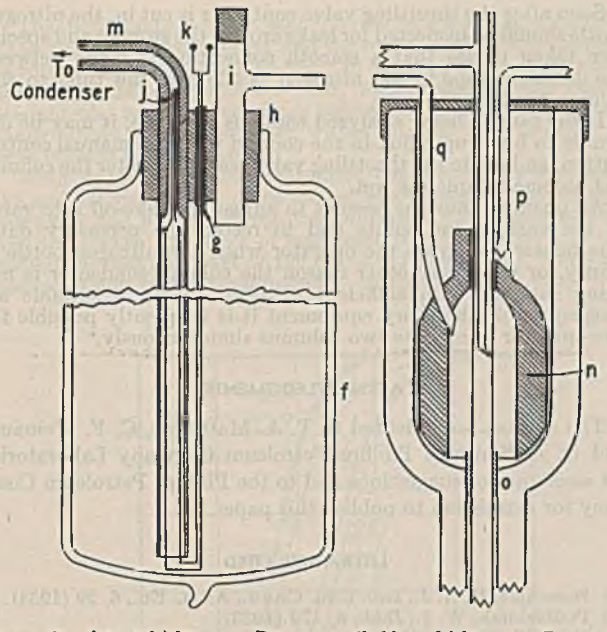

Figure 3. Liquid Nitrogen Dispenser (left) and Nitrogen Expander (right)

f. Dewar flask
g. Porcelain conduit
h. Rubber stopper
i. Air intake tube
j. Heating element
k. Electrical lead wires
m. Discharge tube
n. Nitrogen expander
o. Asbestos string packing
p. Liquid nitrogen intake tube
q. Spent nitrogen exhaust tube

pressure is required in the flask to force the liquid nitrogen out; this extra pressure is furnished automatically by the valve, since the ball on the needle merely assumes a position further up the throat, further restricting the flow of air. Within reasonable limits, the rate of furnishing compressed air to the nitrogen-dispensing system produces no difficulties to operation, since the needle in the throttling valve automatically adjusts itself to a position suitable for operation at a given rate of air flow. The rate of air flow is usually set to permit the ball of the needle to operate at a point about 1 to 1.5 cm. below the narrowest section of the throat. Since the nitrogen expander has been designed to permit rapid response of column pressure to the addition of cooling agent, the throttling valve is capable of automatically controlling the column pressure within very narrow limits of fluctuation.

LIQUID NITROGEN DISPENSER

The nitrogen-dispensing unit shown in detail in Figure 3 (left) is contained in a quart-size, wide-mouthed Dewar flask. An air intake tube, *i*, a nitrogen discharge tube, *m*, and electrical leads, *k*, are sealed into the flask by means of a rubber stopper, *h*, and a porcelain conduit, *g*, for the electrical lead wires. Air which has been induced to flow into the Dewar flask as a result of the action of the throttling valve will force liquid nitrogen up *m* into contact with the heating element, *j*, which being relatively hot flash-vaporizes a small portion of the liquid nitrogen, and the sudden expansion of the vapor forcibly ejects the liquid nitrogen from the discharge end of the tube. The increments of liquid nitrogen discharged in this manner are of small size, since the flash-vaporization occurs before any large quantity can find its way into the upper portion of the discharge tube. The heating element in the discharge tube will continue to expel nitrogen in small bursts, at rapid, regular intervals, which are regulated as regards amount of nitrogen and time interval by the pressure brought to bear by the throttling valve, which in turn reflects the need of the column for cooling agent.

The heating element can be made from 8.75 cm. (3.5 inches) of No. 26 Chromel resistance wire, sufficient to make a heater which extends from just below the rubber stopper to the bend at the top of the discharge tube. During an analysis it is necessary to vary the amount of current in the heating element; this can be done with a small 2-ohm, 5-ampere rheostat if a potential of 6 to 9 volts is being used. The discharge tube, *m*, should be made of 9-mm. glass tubing sealed as it enters the rubber stopper to capillary tubing having 2.5- to 3-mm. inside diameter. Capillary tubing smaller than 2.5 mm. cannot be used successfully, as the large resistance offered to the flow of nitrogen makes it necessary to maintain an unduly large pressure in the Dewar flask in order to force over sufficient nitrogen.

The heating element in the liquid nitrogen dispenser is useful for reducing the fluctuation in column pressure during the fractionation of methane and ethane, since it tends to cause delivery of the cooling agent to the column head in the form of a more nearly continuous stream of dropwise increments of liquid nitrogen automatically adjusted to meet the column requirements. For separating the components propane through hexane the throttling valve usually affords satisfactory control of column pressure. The use of the heating element in the liquid nitrogen dispenser appears to cause very little if any significant increase in the liquid nitrogen requirements for fractionating methane and possibly ethane. Since only a small portion of the liquid nitrogen is vaporized by the heater while fractionating methane,

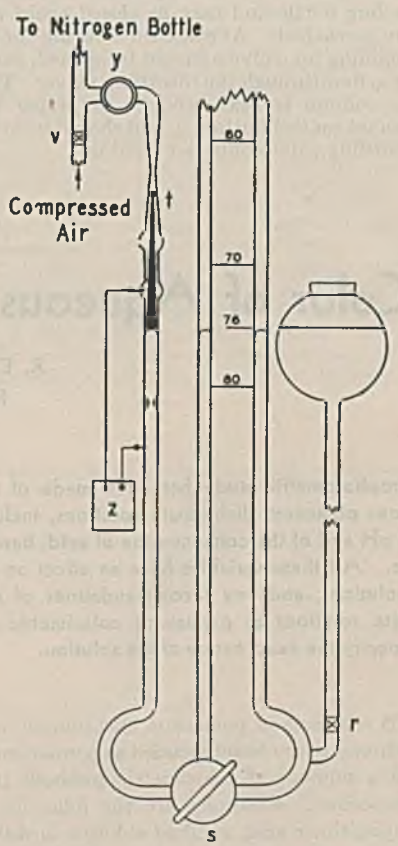

Figure 4. Connection of Throttling Valve to Column Manometer

r. Pinch clamp
s. Three-way stopcock
t. Throttling valve
v. Valve on compressed air line
y. Manual control button
z. Electrical buzzer

the more uniform manner of dispensing nitrogen to the column head enables better operation of the column and more efficient use of the cooling agent, thereby partially compensating for the nitrogen requirements of the heater.

NITROGEN EXPANDER

In designing the nitrogen expander (Figure 3, right) and the nitrogen intake tube, it is important to keep in mind that the liquid nitrogen should be conducted from the Dewar flask to make contact with the distilling tube by the shortest possible

route and should in no case pass through a difficult path before making contact. The intake tube, *p*, should be a continuation of tube *m* in the nitrogen flask, and should have the same inside diameter (2.5 mm.). Expander *n* is machined from a solid piece of metal, and is supported by small pieces of rubber or felt inside the column condenser. This type of expander allows the nitrogen to contact the distilling tube immediately, and conducts any excess nitrogen away from the distilling tube where it may vaporize without causing excessive cooling near the tube. The packing, *o*, is asbestos string wound around the distilling tube and pressed into place. Precautions must be taken to prevent water from accumulating in the nitrogen expansion chamber or around the distilling tube.

TECHNIQUE OF OPERATION

During the time that a sample is introduced into the fractionating column, the throttling valve controller should be cut out by turning the stopcock (*s*, Figure 4). After sampling is completed and the kettle allowed to warm up, clamp *r* should be released from the leveling bottle and may be closed again when the desired pressure is reached. At some time during the period when pressure is building up, valve *v* should be opened, causing a small stream of air to flow through the throttling valve. The condenser head on the column is next brought to proper temperature, using the manual control button, *y*, and should be at temperature when the throttling valve controller is cut in.

Soon after the throttling valve controller is cut in, the nitrogen bottle should be inspected for leaks around the stopper and special care taken to see that a smooth connection is made between the discharge tube in the nitrogen bottle and the tube to the column head.

If the sample being analyzed contains methane, it may be desirable to build up reflux in the column with the manual control button, and cut in the throttling valve controller after the column has become completely wet.

An operator must be present to adjust the take-off rate valve at the various cut points and to record the necessary data. The buzzer, *z*, signals the operator when the nitrogen bottle is empty, or if for any other reason the column condenser is not being supplied with sufficient nitrogen. With a suitable arrangement of laboratory equipment it is frequently possible for one operator to operate two columns simultaneously.

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Color of Aqueous Potassium Dichromate Solutions

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A spectrophotometric study has been made of the color of aqueous potassium dichromate solutions, including the effect of pH and of the concentration of acid, base, and dichromate. All these variables have an effect on the color of the solutions, and any recommendations of potassium dichromate solutions as permanent colorimetric standards should specify the exact nature of the solution.

AQUEOUS solutions of potassium dichromate and/or potassium chromate are recommended as permanent colorimetric standards in a number of colorimetric methods involving unstable yellow colors. Examples are the following procedures: silica as molybdisilicic acid, residual chlorine in water, carotene, and varnish. In spite of these widespread uses, little work has been done on the color of potassium dichromate and potassium chromate solutions, especially the effect on them of such variables as pH and the kind and amount of acid or base added.

Hantzsch and Clark (*3*), Neuss and Rieman (*4*), Sherrill (*5*), Vosburgh and Cooper (*7*), and others have studied the chromate-dichromate relationship from the standpoint of the ionic equilibria involved rather than the color of the solutions. Swank and Mellon (*6*) pointed out the necessity of buffering potassium chromate solutions at pH 9 to secure color matches with molybdisilicic acid.

The present spectrophotometric study was undertaken to determine the effect of pH, various acids and bases, and dichromate concentration on the color of aqueous potassium dichromate solutions.

EXPERIMENTAL WORK

Apparatus and Solutions. Transmittancy measurements were made in 1.000-cm. cells with a General Electric recording spectrophotometer, adjusted for a spectral band width of either 5 or 10 mμ. In case of colored reagents, these solutions were used in the reference cell. Otherwise, redistilled water served. All pH measurements were made with a glass electrode assembly.

Stock solutions of potassium dichromate were prepared from twice recrystallized salt and redistilled water. Two solutions, containing 10.00 grams per liter and 12.50 grams per 100 ml., were used. A series of Clark and Lubs buffer solutions was prepared according to the directions of Clark (*2*).

Effect of pH on the Color of Potassium Dichromate Solutions. To study the effect of pH on the chromate-dichromate system, the desired amount of potassium dichromate solution was pipetted into a 50-ml. volumetric flask, and then diluted to the mark with a buffer of the desired pH. The contents were mixed and allowed to sit a few minutes to ensure equilibrium. The spectral transmission curve and the pH were then determined.

In general, dichromate solutions at a low pH have an orange hue, while those at a high pH are yellow. These colors are commonly associated with the dichromate and chromate ions, respectively. An intermediate range exists in which the hue is extremely sensitive to small changes in pH. At low dichromate concentrations this intermediate range extends from pH 5 to 7, as shown in Figure 1. At higher concentrations, the range shifts upward, being from pH 6 to 8 at 2.0 mg. of potassium dichromate per ml.

At low concentrations of salt the series of solutions at various pH values has an isobestic point (*1*) (Figure 1). With different concentrations of dichromate, the transmittancies of this point vary according to Beer's law. The wave length at this point decreased from 444 mμ at 0.2 mg. to 440 mμ at 1.0 mg. of dichromate per ml. At concentrations greater than 1.0 mg. per ml. the absorption is too high at these wave lengths to show the point.

Effect of Acids. In studying the effect of various acids, and in all subsequent work, the following procedure was used:

The dichromate solution was pipetted into a 50-ml. volumetric flask, the acid or base added, and the solution diluted to the mark at room temperature with water. In case the water could not be added to the concentrated acid, approximately the desired amount was added to the flask before the acid. The transmittancy curves were determined within an hour after making the final volume adjustments.

With single acids two dichromate concentrations were used: 0.4 and 5.0 mg. per ml. The former was low enough to show the relatively flat portion of the transmittancy curve near 440 mμ, and to observe the effect of acid upon it. The latter was high enough so that this part of the curve did not appear. The acid concentration varied from none to concentrated acid.

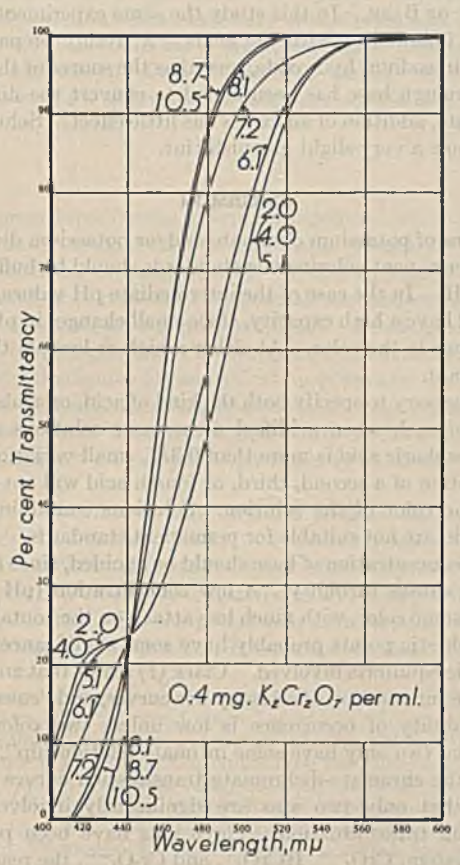

Figure 1. Effect of pH on the Color of Potassium Dichromate Solution
pH on curve

With mixed acids only one concentration of dichromate was used: 0.4 mg. per ml. The total acid concentration did not exceed that likely to be encountered in general analytical practice. With the binary mixtures 5 ml. of each acid per 50-ml. final volume was the maximum, while with the ternary and quaternary mixtures the maximum of each acid was limited to 3 ml.

Lack of space prevents presentation or adequate description of all the transmittancy curves. An attempt is made in the following paragraphs to summarize their characteristics:

Sulfuric Acid. Potassium dichromate solutions containing sulfuric acid show the greatest variation in color of any of the acids used. With a low concentration of dichromate, small amounts of acid give a very definite minimum in the curve near 440 mμ, the wave length of the minimum being related to the acidity (Figure 2). Larger amounts of acid, up to 9*M*, cause no great change. Between 9 and 11*M*, however, the hue of the solutions changes from orange to orange-red, and the minimum point in the curve disappears. As the acidity is increased further, there is a shift toward an orange-brown hue, with no striking change in the curve.

A high concentration of dichromate behaves similarly, but there is no minimum in the curve at concentrations much greater than 1 mg. of dichromate per ml. because of complete absorption where the minimum should be. Even a small amount of acid shifts the entire curve toward longer wave lengths. Then acidities on up to 9*M* have little effect on the color; but between 9 and 12*M* the color changes from orange-red to brown. Above 12*M* there is little further color change.

Nitric Acid. With a low dichromate concentration solutions containing nitric acid show the first of the two color changes noted with sulfuric acid–potassium dichromate solutions. The minimum near 440 mμ appears when the acidity reaches 5*M*, and the intensity of the minimum increases with increasing acidity. With a high dichromate concentration the entire curve shifts to longer wave lengths as the nitric acid concentration increases. The brown color noted with sulfuric acid does not appear.

Phosphoric Acid. Qualitatively phosphoric acid has much the same effect on the dichromate color as nitric acid, but quantitatively it differs considerably. With a low dichromate concentration the minimum near 440 mμ is definite with 0.3*M* acid. Increasing the acidity to 1.0*M* increases the intensity greatly, but higher acidities cause little further change. The transmittancy curve for high dichromate concentrations shifts to longer wave lengths with small amounts of acid, but, as before, amounts greater than 1.0*M* have little additional effect.

Perchloric Acid. With a low dichromate concentration, this acid has little effect on the color. The minimum in the transmittancy curves does not appear, but the percentage transmittancy of the horizontal portion of the transmittancy curve increases slightly with increased acid concentration. In the presence of larger amounts of potassium dichromate, a precipitate, presumably potassium perchlorate, appears.

Acetic Acid. Dichromate solutions containing acetic acid show changes similar to those with nitric acid. The magnitude of the changes is greater than those with nitric acid, although not so large as the corresponding changes with sulfuric acid.

Hydrochloric Acid. Although it is generally assumed that potassium dichromate does not oxidize hydrochloric acid in aqueous solution, the yellow color of solutions which contain 0.4 mg. of potassium dichromate per ml. and which are 9*M* or stronger in acid disappears within 5 minutes after addition of the acid. Because of this reaction, which proceeds at a slower rate in more weakly acidic solutions, hydrochloric acid solutions of potassium dichromate fade and should not be used as permanent

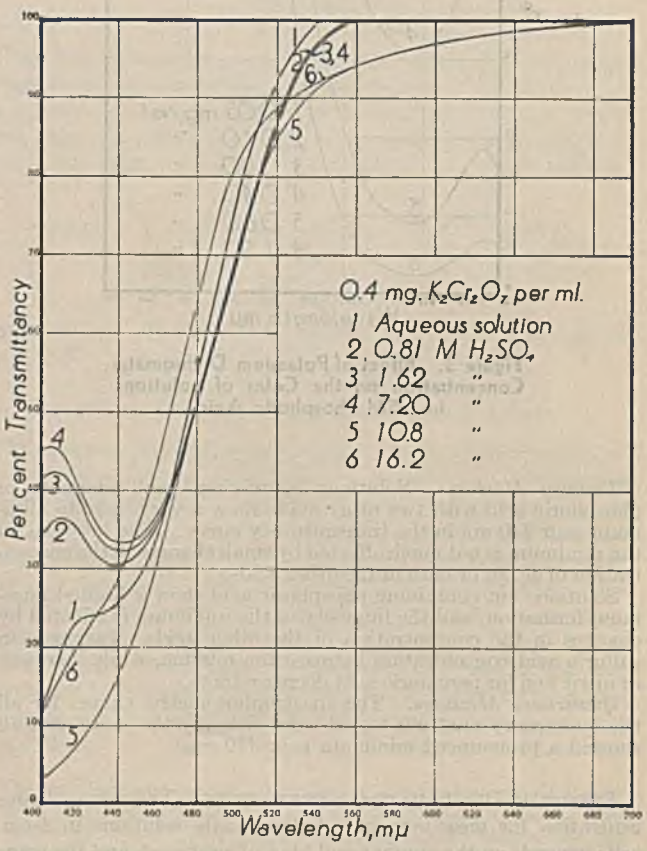

Figure 2. Effect of Sulfuric Acid on the Color of Potassium Dichromate Solutions

standards. A solution 0.8*M* in acid fades about 2 per cent in 48 hours, and one 0.4*M* in acid fades to the same extent in a month.

If the fading of the solution is overlooked, hydrochloric acid affects the color in a manner similar to nitric acid. The minimum appears at 0.4*M* acid, and as the acid concentration increases the minimum is more marked.

Binary Mixtures. Binary mixtures containing small amounts of phosphoric acid and one of the other acids show very definite minima near 440 mμ. Changes in the concentration of the second acid have only a small effect on the color of the solution. The minimum is present in the transmittancy curve even if the second acid is nitric or perchloric acid, neither of which, alone and at low concentrations, causes the minimum to appear.

Mixtures of either perchloric or nitric acid and sulfuric acid show limited minima formation at low, and definite minima at higher, sulfuric acid concentrations.

Mixtures of nitric and perchloric acid show no minima in the transmittancy curve.

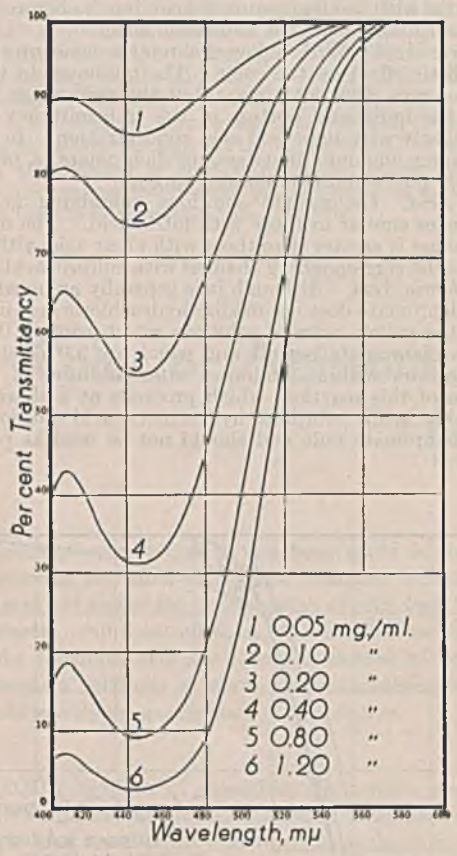

Figure 3. Effect of Potassium Dichromate Concentration on the Color of Solutions in 1.5M Phosphoric Acid

Ternary Mixtures. Solutions containing small amounts of phosphoric acid with two other acids show a very definite minimum near 440 mμ in the transmittancy curve. The intensity of the minimum is not much affected by small changes in the concentration of either or both of the other acids.

Solutions not containing phosphoric acid show a limited minimum formation, and the intensity of the minimum is affected by changes in the concentration of the other acids. Increases in sulfuric acid concentration increase the minima, while increases in nitric and/or perchloric acid decrease them.

Quaternary Mixtures. The spectrophotometric curves for all the quaternary mixtures tested were virtually the same, and all showed a pronounced minimum near 440 mμ.

Effect of Dichromate Concentration. The range of concentration for measurements of dichromate solutions in 1-cm. cells depends on the amount and kind of acid used, and the wave length at which the measurements are made. With 1-cm. cells, measured at the wave length of minimum transmission, 0.02 to 1.2 mg. of potassium dichromate per ml. can be determined in solutions 1.8*M* in sulfuric acid or 1.5*M* in phosphoric acid (Figure 3). Beer's law is valid over the entire range in both cases. With 1.6*M* nitric acid, 1.7*M* acetic acid, or 0.9*M* perchloric acid, 0.02 to 0.9 mg. of potassium dichromate per ml. can be determined if the measurements are made in 1-cm. cells at 430 mμ. Beer's law is valid only to 0.6 mg. per ml. for these solutions.

If the dichromate solution is adjusted to pH 2 or 9, and the transmittancy measurements are made in 1-cm. cells at 440 mμ the measurable range is 0.02 to 1.0 mg. per ml., Beer's law being valid over the entire range.

Effect of Base. In this study the same experimental procedure was followed as with the acids. A freshly prepared solution, 8*M* in sodium hydroxide, served as the source of the base.

After enough base has been added to convert the dichromate to chromate, addition of an excess has little effect. Solutions 8*M* in base show a very slight greenish tint.

DISCUSSION

Solutions of potassium chromate and/or potassium dichromate used as permanent colorimetric standards should be buffered to a definite pH. In the case of the intermediate pH values, the buffer should have a high capacity, since small changes in pH make a large change in the color. At either a high or low pH this is not so important.

It is necessary to specify both the kind of acid, or acids, and the amount of each when acidified dichromate solutions are used. If the phosphoric acid is more than 0.3*M*, small variations in the concentration of a second, third, or fourth acid will not seriously change the color of the solution. Solutions containing hydrochloric acid are not suitable for permanent standards.

A high concentration of base should be avoided, since it attacks glass and causes turbidity. A low concentration (pH 9 or 10) gives the same color, with much less attack on the container.

The isobestic points probably have some significance in terms of the ionic equilibria involved. Clark (*1*) states that an isobestic point is an intersection of all isohydric curves, and "consequently the probability of occurrence is low unless two colored compounds and two only have some intimate relationship". Such a point in the chromate-dichromate transmission curves seems to indicate that only two ions are significantly involved in the equilibrium transformation. Three ions have been postulated for this system, CrO_4^{--}, $HCrO_4^-$, and $Cr_2O_7^{--}$, the reactions involved being

$$CrO_4^{--} + H^+ \rightleftharpoons HCrO_4^- \quad (1)$$

$$2HCrO_4^- \rightleftharpoons Cr_2O_7^{--} + H_2O \quad (2)$$

The isobestic point would seem to indicate that Reaction 1 is of major importance, since it alone involves hydrogen ions. Calculations from the data of Neuss and Rieman (*4*) show that 88 per cent of the dichromate ion in a solution containing 1 mg. of potassium dichromate per ml. should react to give the $HCrO_4^-$ ion. If these data, and the facts about isobestic points, are correct, the absorption spectra of the $HCrO_4^-$ and $Cr_2O_7^{--}$ ions should be similar, since conversion of one form to the other has little effect on the over-all transmittancy.

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Gravimetric Determination of Tungsten

With Anti-1,5-di-(p-methoxyphenyl)-1-hydroxylamino-3-oximino-4-pentene

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A new organic compound has been developed as a reagent for the gravimetric determination of tungsten. Its physical and chemical properties have been investigated and procedures are given for its use in the determination of tungsten in ores and alloys. Determinations of tungsten with the new reagent are equivalent in accuracy to the standard cinchonine method.

MOST procedures for the gravimetric determination of tungsten involve separation of the major portion of the element from solution as tungstic acid, H_2WO_4, by strong mineral acid treatment, and recovery of the small amount remaining in solution by means of an organic precipitant. Cinchonine is generally used for this purpose. Present governmental restrictions on the sale of cinchonine for analytical purposes and the increasing number of applications of tungsten and tungsten alloys to industrial uses make the introduction of an effective and easily obtainable reagent for tungsten particularly desirable at this time.

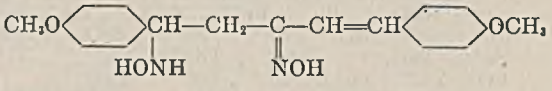
This paper describes a new organic reagent for tungsten, anti -1,5 - di - (*p* - methoxyphenyl) - 1 - hydroxylamino - 3 - oximino-4 pentene, first synthesized in this laboratory and applied to the analysis of a variety of tungsten ores and alloys with highly satisfactory results. The compound may now be obtained from LaMotte Chemical Products Co., Towson 4, Baltimore, Md.

Various organic reagents for the gravimetric determination of tungsten have been proposed since Lefort (*13*) first reported in 1881 that quinine acetate would precipitate tungstates from solution. Cremer (*1*) reported the cinchonine reaction in 1895, but it was not applied to actual analyses until about 20 years later when Low (*14*) developed a gravimetric procedure employing cinchonine as a precipitant for tungsten. Jannasch and Bettges (*8*) used hydrazine hydrochloride and strong hydrochloric acid to precipitate tungsten but separations were not complete by this method. Knorre (*11*) found benzidine hydrochloride slightly better than hydrazine hydrochloride but still not entirely satisfactory.

Other investigators have proposed a variety of gravimetric organic reagents: α-naphthylamine and cumidine (*23*), tetramethyl-*p*-diaminodiphenylmethane (*10, 20*), 1,4-diphenylendanilodihydrotriazole (Nitron) (*4*), quinoline (*15*), tannin (*16, 21*), phenylhydrazine hydrochloride (*2*), 8-hydroxyquinoline (*5, 9, 18*), vanillylidene-benzidine (*7*), and rhodamine B (*17*). None of these reagents has proved sufficiently effective to replace cinchonine as the preferred reagent in standard procedure, although its use involves certain difficulties (*1, 6, 12*).

Early in 1931 a systematic investigation of the reactivity of organic compounds, with respect to color and precipitate formation with inorganic ions, was begun in this laboratory. Standard solutions of about eighty inorganic ions were prepared and tested, in both acid and alkaline medium, with various types of organic compounds, especially those with one or more chelating or salt-forming groups. The oximes, as a class, appeared to be one of the most promising types to try in this search for specific and highly sensitive reagents for inorganic ions.

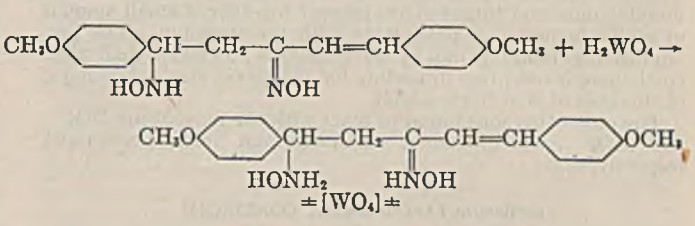
During the 1930's several new and important organic analytical reagents were discovered in this laboratory (*19, 24–27*). In 1940 anti - 1,5 - di - (*p* - methoxyphenyl) - 1 - hydroxylamino - 3 - oximino-4-pentene, one of a series of compounds synthesized expressly for this work, was observed to form a highly insoluble yellow precipitate with tungstate ions (WO_4^{--}) in acid solution. A detailed investigation revealed that this new compound combines with the tungstate ion



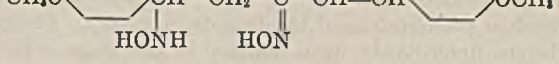
in a definite ratio of one molecule of reagent to one tungstate ion and that this reaction may be applied to the gravimetric determination of tungsten.

NATURE OF REACTION WITH TUNGSTATE ION

The exact mechanism by which the reagent combines with the tungstate ion to form the insoluble complex is not known with certainty. It is possibly a salt-forming reaction:



The reagent belongs to the basic salinogenic group of organic compounds, many members of which combine with anions such as nitrates, perchlorates, sulfate, phosphates, molybdates, vanadates, thiocyanates, etc. (*28*), in acid medium. The "syn" isomer of the organic compound is completely unreactive with the



tungstate ion. It crystallizes in the form of colorless plates (m.p. 217° C.).

PHYSICAL AND CHEMICAL PROPERTIES OF REAGENT

The reagent crystallizes from ethanol in the form of small yellow needles which melt at 156–157° C. (corrected). It is soluble in the following organic solvents, solutions being yellow in all instances: ethanol, acetone, ethyl ether, ethyl acetate, dioxane, acetic acid, and benzene. The compound is insoluble in water and petroleum ether. Solutions of the reagent in ethanol are used in tungsten analyses. The solubility is 0.766 gram of reagent per 100 ml. of ethanol at 25° C.

SOLUBILITY OF TUNGSTEN COMPLEX. An experiment was conducted to determine whether or not the precipitated organotungsten complex was soluble in water to the extent of one part per million.

One milligram of finely pulverized dried complex (dried at 105° C. for 2 hours) was introduced into a flask containing 1 liter of water at 25° C. The water was mechanically stirred for one week. At the end of that time, the complex had not dissolved; hence it was concluded that its solubility is less than 1 mg. per liter of water—i.e., 1 p.p.m.

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STABILITY OF REAGENT TO LIGHT. The pure reagent is pale yellow in color but is slowly darkened by light. This discoloration does not affect its reactivity with tungstates. No discoloration occurs if stored in dark bottles.

REACTIONS WITH INORGANIC IONS Tests for reactivity with inorganic ions were made on porcelain spot plates by adding a drop of an ethanol solution of the reagent to a drop of solution containing the respective ions (approximately 0.05 mg.) in both acid and alkaline medium where possible. No reactions were observed between the reagent and any of the following ions:

Ag^{+}, Al^{+++}, As^{+++}, AsO_4^{---}, $B_4O_7^{--}$, Ba^{++}, Be^{++}, Bi^{+++}, Br^{-}, CO_3^{--}, Ca^{++}, CbO_4^{---}, Cd^{++}, Ce^{+++}, Cl^{-}, Co^{++}, Cr^{+++}, Cs^{+}, Dy^{+++}, Er^{+++}, Eu^{+++}, F^{-}, Fe^{++}, Ga^{+++}, Gd^{+++}, Ge^{++++} (aqueous solution of GeO_2), HfO^{++}, Hg^{+}, Hg^{++}, I^{-}, In^{+++}, K^{+}, La^{+++}, Li^{+}, Mg^{++}, Mn^{++}, NO_2^{-}, NO_3^{-}, Na^{+}, Nd^{+++}, Ni^{++}, HPO_4^{--}, Pb^{++}, Pr^{+++}, $PtCl_6^{--}$, Rb^{+}, ReO_4^{-}, Rh^{+++}, Ru^{+++}, S^{--}, SO_4^{--}, Sb^{+++}, Sc^{+++}, SeO_3^{--}, SiO_3^{--}, Sm^{+++}, Sr^{++}, TaO_4^{---}, TeO_4^{--}, TiO^{++}, Th^{++++}, Tl^{+++}, Tm^{+++}, VO^{+}, Y^{+++}, Yb^{+++}, Zn^{++}, Zr^{++++} (ZrO^{++}).

Auric, ceric, and iridic ions produce brown precipitates with the reagent but these are extremely rare in tungsten ores and alloys.

Ferric iron reacts to form a small amount of brown precipitate but not enough to interfere even in the analysis of steels containing tungsten.

Stannous and stannic tins produce an orange and a yellow precipitate, respectively, with the reagent.

Copper reacts in alkaline solution to produce a brown precipitate but does not react at all in acid medium. In analysis, the reagent is always used in acid solution; hence copper presents no interference.

Molybdates are precipitated but not quantitatively. When molybdenum and tungsten are present together, a small amount of molybdenum may precipitate with the tungsten. The precipitation of molybdenum by the reagent is no greater than when cinchonine is used (see procedure for analysis of steels and alloys; cf. analysis of N.B.S. steel 132).

The only other ions found to react with the reagent are UO_2^{++} and OsO_5^{--}, which produce an orange and brown precipitate, respectively.

OPTIMUM EXPERIMENTAL CONDITIONS

PERMISSIBLE ACIDITY. Tungsten is quantitatively precipitated by the reagent when the acidity is less than pH 1. Partial precipitation occurs above pH 1 and above pH 5 no precipitation occurs. The best acidity to use is about 0.2*N* hydrochloric acid. Sulfuric and nitric acids may also be used, but in the presence of lead or tin sulfuric acid is objectionable and nitric acid solutions stronger than 1*N* begin to attack the reagent.

TEMPERATURE OF SOLUTION. Room temperature is preferable for effective precipitation of the tungsten complex. Precipitation begins immediately upon addition of the reagent in cold solution, with the precipitate collecting and settling much more readily than if the solution is hot. The reagent should therefore not be added to hot solutions.

AMOUNT OF REAGENT REQUIRED. The reagent combines with tungstate ions in a ratio of 1 to 1. However, when the approximate quantity of tungsten is known, it is recommended that twice the theoretical amount of reagent be added to ensure complete and rapid precipitation. If the tungsten content is unknown, the amount of reagent required may be judged by observing the color of the precipitated complex as it forms.

TIME NECESSARY FOR COMPLETE PRECIPITATION. Complete precipitation is obtained when solutions are allowed to stand at room temperature for 3 hours after the addition of excess reagent, with occasional stirring to collect the precipitate. Allowing solutions to stand overnight ensures complete precipitation but is not necessary.

WASHING THE PRECIPITATE. The most effective wash solution has been found to be a cold, dilute hydrochloric acid solution containing a small amount of the reagent. It is prepared by diluting 20 ml. of concentrated hydrochloric acid to 1 liter and adding 1 ml. of a saturated ethanol solution of the reagent.

FINAL TREATMENT OF PRECIPITATE. Attempts to weigh the organo-tungstate precipitate directly, after washing and drying, indicate that the method is impractical. Conditions have to be tediously controlled to get consistent results. The difficulties arise from the fact that the reaction must occur in acid solution and that the reagent itself precipitates when added in small excess. Unless the reagent is added immediately after acidifying the solution, some tungsten may precipitate as tungstic acid and be occluded in the organic precipitate, preventing a consideration of it as a compound of definite composition. It is also difficult to remove precipitated reagent from the complex without at the same time dissolving some of the complex.

The complex may be ignited easily to tungstic oxide, WO_3, in which case it makes no difference if tungstic acid and excess reagent are present, because tungstic acid is converted to tungstic oxide and excess reagent is completely removed upon ignition

The precipitated complex is easily and rapidly separated by filtration through quantitative ashless filter paper. Whatman filter paper No. 40 and other papers of similar texture and grade are suitable for quantitative analyses when using this reagent. Filtration is most rapidly accomplished if the precipitated complex is allowed to settle (about 3 hours is sufficient) and the solution filtered by decantation. There is very little tendency for the precipitate to clog the pores of the paper, thus permitting thorough washing with a minimum time consumption.

The washed precipitate may be ignited without difficulty. There is no tendency for explosion, or decomposition rapid enough to sweep out any of the contents of the crucible. The precipitate and the filter paper decompose at about the same rate. Moisture should be removed at about 100° C. before raising the temperature of the crucible and contents to ignition temperature. The crucible should not be heated above a dull red until all carbon has been burned off.

DETERMINATION OF TUNGSTEN IN SOLUTIONS, STEELS, ALLOYS, AND ORES

Of the various procedures investigated, a modification of that of Hillebrand and Lundell (*6*, pp. 553–5) has been found most satisfactory for the analysis of tungsten ores. For steels and tungsten alloys, modifications of the procedures of the American Society for Testing Materials (*22*, pp. 1011–2) are both speedy and accurate.

DETERMINATION IN ORES. Transfer to a 400-ml. beaker 1 gram of sample which has been ground in an agate mortar to 200-mesh or finer and dried to constant weight at 105° C. Unless the sample is ground to 200-mesh or finer, a protective layer of precipitated tungstic acid may coat the particles, preventing the hydrochloric and nitric acids from coming in contact with all the unreacted material. This is especially true with ferberite and wolframite, which are difficult to decompose. If sufficient hydrochloric acid is used and the temperature is held at approximately 75° C., the formation of tungstic acid on the particles may be avoided. Serious error may be introduced by this effect if samples of particle size much larger than 200-mesh are used. The magnitude of the error depends on the mineral being analyzed. Scheelite, $CaWO_4$, and hubnerite, $MnWO_4$, are easily decomposed by the hydrochloric-nitric acid treatment, in which case the error is small; ferberite, $FeWO_4$, and wolframite, $(Fe,Mn)WO_4$, being much more difficult to decompose, require very small particle size.

Add 5 ml. of distilled water and rotate the beaker so as to distribute the sample evenly over its bottom. Add 100 ml. of hydrochloric acid (sp. gr. 1.19), cover the beaker with a watch glass, and heat for one hour at a temperature not exceeding 60° C. Stir occasionally to break up formations of crusts and to facilitate contact of the acid with all particles of the sample. Raise the watch glass on glass hooks and cautiously boil to a volume of about 50 ml. Break up the material on the bottom of the beaker with a glass stirring rod. Add 40 ml. of hydrochloric acid and 15 ml. of nitric acid and again boil the solution to about 50 ml. Stir up the caked matter again, add 5 ml. of nitric acid, and boil to 10 or 15 ml. Dilute to 250 ml. with hot water and heat just below boiling for 30 minutes. Allow to cool to room temperature and add 25 ml. of alcoholic reagent solution (made by dissolving 0.7 gram of reagent in 100 ml. of ethanol) slowly with constant stirring. Allow the precipitate to settle for about 2 hours and test the supernatant liquid with a few drops of the reagent for complete precipitation of the tungsten.

Because of the limited solubility of the reagent in aqueous solutions, some precipitate may be expected to form whenever the reagent is added. The appearance of the precipitate formed in the presence of tungsten is different from that formed when the latter is absent. The organic tungstate is deep yellow-orange in color while the precipitated reagent alone is almost white. It is advisable for an analyst using the reagent for the first time to add some of it to a solution of sodium or ammonium tungstate (1 mg. of tungstate per ml.) acidified with 8 or 10 volumes of 0.2*N* hydrochloric acid. The same amount of reagent should be added to a similar volume of 0.2*N* acid alone. The striking difference between the color of the reagent and that of the organo-tungstate may be observed by this simple procedure.

In testing a solution for completeness of precipitation of tungsten, more reagent should be added if an orange precipitate is formed. If it is white or pale yellow, precipitation of the tungsten is complete.

After the precipitate has settled at least 2 hours (or overnight if convenient) filter by decantation through an 11-cm. ashless filter paper. Wash the precipitate several times with reagent wash solution.

Transfer the precipitate to an ashless filter paper and moderately scrub the beaker by means of a rubber policeman to remove, as far as possible, any precipitate adhering to the walls of the beaker. It is not necessary at this point to attempt to remove all the finely divided tungstic acid adhering to the walls of the beaker, since the main precipitate, after it is washed, is to be transferred back to this beaker and all the precipitated tungsten redissolved.

Wash with the prepared wash solution. Repeat several times and set aside the combined filtrate and washings. Test the filtrate with a few drops of reagent solution for complete precipitation of the tungsten. It is rare that any is found in this filtrate. Transfer the filter paper containing the washed precipitate to the original beaker and add 6 ml. of concentrated ammonium hydroxide. Shred the filter paper to a uniform pulp by means of a glass stirring rod, cover the beaker, and warm gently for a few minutes. Stir the pasty mass with the rod, then wash down the inside of the beaker with warm dilute ammonium hydroxide (1 to 9) containing 10 grams of ammonium chloride per liter. Warm again and stir thoroughly. Filter through an 11-cm. ashless filter paper and collect the filtrate in a 400-ml. beaker. Wash the original beaker and residue several times with the warm dilute ammonium hydroxide solution; between washings, squeeze as much of the liquid from the fibers of the pulp as possible. Wash the beaker and residue with several small portions of hot 95 per cent ethanol to dissolve any organo-tungstate that has not been decomposed by the ammonium hydroxide treatment. Follow with a final washing with the warm dilute ammonium hydroxide solution. Keep the volume of liquid as small as possible. Reserve the residue of filter fiber for further recovery of traces of tungsten.

Evaporate the filtrate to a volume of about 50 ml.; add 20 ml. of concentrated hydrochloric acid and 10 ml. of concentrated nitric acid; cover and cautiously boil to a volume of 10 to 15 ml. If any organic residue is still present and tends to adhere to the walls of the beaker, it may be decomposed by the addition of more hydrochloric and nitric acids in the same ratio as above. Dilute the solution to about 250 ml. with water and allow it to cool slowly to room temperature. Add the alcoholic reagent solution until the color of the precipitate, as it forms, indicates complete precipitation of the tungsten. Allow the precipitate to settle and filter through an 11-cm. ashless filter paper; wash thoroughly with reagent wash solution. If the filtrate has a clear yellow tint, precipitation is complete. If it is colorless, more reagent must be added to complete the precipitation. The washed precipitate is the main precipitate and is to be ignited with the very small amounts of tungsten that may be obtained in the procedure described in the following paragraphs.

Any tungsten that may not have been recovered is contained in the reserved residue of filter fiber, in combination with iron or alumina or with small amounts of reagent that were not completely dissolved. This combined tungsten may be dissolved by digesting the filter paper and residue of fiber with warm dilute hydrochloric acid (1 to 9). Filter and wash the residue with small amounts of hot 0.5 per cent ammonium chloride solution and the warm dilute ammonium hydroxide wash solution, collecting all in the same vessel. Acidify the filtrate with hydrochloric acid until it is approximately 0.2*N* and then slowly add 5 to 10 ml. of alcoholic reagent solution. Any precipitate obtained should be filtered and washed with the reagent wash solution. Reserve the washed precipitate and ignite later with the main one already obtained.

The residue that now remains is usually free from tungsten. To be positive, ignite it in a porcelain crucible (not a platinum crucible because tin might be present), transfer the ash to a platinum crucible, and volatilize the silicon by treating it with hydrofluoric and sulfuric acids. Fuse the remaining residue with as little sodium carbonate as possible, cool, and extract the melt with water. Filter and acidify the filtrate with hydrochloric acid, boil to expel carbon dioxide, and add dropwise some of the reagent solution to test for the possible presence of tungstate ions. If the precipitate is orange, upon the addition of the first few drops of reagent, filter it off, wash with the reagent wash solution and ignite with the two residues already obtained. (The authors have never obtained any organo-tungstate precipitate at this point.)

Place the papers containing the main precipitate and the two recoveries in a weighed platinum crucible and heat at a temperature below a dull red until all the carbon has been burned off. Cool, add a few drops of hydrofluoric acid to the residue (enough to moisten it completely), add a drop of sulfuric acid and evaporate to dryness over a water or sand bath. Reignite in order to get the weight of WO_3 free from SiO_2.

The tungstic oxide obtained at this point is not pure but must be examined for contaminants. The examination for contaminants does not involve any changes from the standard procedures now in use (*3; 6*, p. 555; *22*). The contaminants most apt to be present at this point are principally iron and molybdenum, with very small traces of phosphorus. The iron is separated by fusion with sodium carbonate, dissolving in hot water, and filtering. Tungsten and molybdenum (if present) form soluble sodium salts. If molybdenum is present, the amount of its contamination may be determined colorimetrically (*3; 22*, pp. 1008–9) in the filtrate. It is rare that the amount of phosphorus present justifies a test for it. The weights of contaminants found are subtracted as oxides from the weight of impure tungstic oxide and the corrected weight is used to calculate the percentage of tungstic oxide or tungsten in the sample.

Determination in Alloys and Metals. Treat 1 gram of the finely divided metallic sample with 5 ml. of hydrofluoric acid in a large covered platinum crucible or dish. After the initial effervescence has ceased, add nitric acid dropwise until the metal has dissolved. Add 15 ml. of sulfuric acid (1 to 1), transfer the vessel to a sand bath, and heat cautiously until dense fumes of sulfur trioxide are evolved freely. Perchloric acid may be substituted for sulfuric acid if desired. In this case, add 15 ml. of perchloric acid (60 per cent) after the hydrofluoric-nitric acid treatment and heat cautiously to dense fumes of perchloric acid. Cool, dilute, and transfer in the same manner as when sulfuric acid is used. The use of perchloric acid shortens slightly the time necessary for this part of the analysis, but otherwise offers no particular advantage.

Allow to cool and transfer the contents to a 400-ml. beaker by washing the platinum vessel with a fine stream of water. Wipe the vessel with a small piece of ashless filter paper and transfer it to the beaker. Rinse the vessel with a little warm ammonium hydroxide (1 to 1), a little water, then a little hot hydrochloric acid (1 to 1). Repeat the treatments with ammonium hydroxide, water, and hydrochloric acid, adding all rinsings to the 400-ml. beaker. Dilute the contents of the beaker with water to about 150 ml. Add 10 ml. of hydrochloric acid (sp. gr. 1.19), cover with a watch glass supported on glass hooks, and boil cautiously for at least 5 minutes. Remove the source of heat and dilute the contents to about 350 ml. with water. Allow to cool and add slowly, with constant stirring, 15 to 20 ml. of alcoholic reagent solution (0.7 gram per 100 ml. of 95 per cent ethanol). Allow the precipitate to settle for about 2 hours and test the supernatant liquid for complete precipitation of the tungsten. If an orange precipitate forms upon the addition of more reagent, precipitation is incomplete. If the precipitate is almost white, separation of tungsten is complete.

When the precipitate has completely settled, filter by decantation through an 11-cm. ashless paper. Wash the precipitate several times with reagent wash solution, ignite the paper and residue, in the platinum vessel in which the sample was treated originally, at a temperature below a dull red heat until all of the carbon is consumed. Add a few drops of nitric acid and evaporate to dryness on a water or sand bath. Ignite to constant weight (preferably in an electric muffle furnace) at a temperature not exceeding 750° C. This is the weight of impure tungstic oxide. Add about 5 grams of sodium carbonate and carefully heat until a clear melt is obtained. Rotate the fused mass in the vessel until it solidifies around the wall of the container. When cool, dissolve the melt in hot water, filter through an 11-cm. ashless paper, and wash thoroughly with hot water. Place the filter in

the crucible and ignite again. Repeat the sodium carbonate fusion on the small residue, using a proportionately smaller amount of carbonate than in the first fusion. Cool and dissolve in the same manner as before. Filter and wash thoroughly to remove all traces of sodium carbonate. Ignite in the same platinum vessel, cool, and weigh. Subtract the weight of this oxide residue from that of the original impure tungstic oxide. Calculate the percentage of tungsten from the corrected weight of tungstic oxide.

If molybdenum is present, the amount of its contamination of the tungsten precipitate can be determined in the sodium carbonate extracts. This is best done colorimetrically (*3; 22*, pp. 1008–9).

ANALYSIS OF STANDARD TUNGSTATE SOLUTIONS

A standard tungstate solution was prepared from C.P. tungstic acid (H_2WO_4, 99.84 per cent pure) by fusion with sodium hydroxide in a silver crucible. The fusion mass was dissolved in water and diluted to the mark in a volumetric flask. Measured volumes of this solution were taken and acidified to 0.2*N* with hydrochloric acid and the tungsten was precipitated by the addition of an ethanol solution of the new reagent. In order to compare the two reagents, the tungsten was also precipitated by cinchonine from a similar series of solutions. The precipitated complexes were filtered off through quantitative paper and ignited in platinum crucibles to tungstic oxide (Table I).

APPLICATION OF PROCEDURES

ANALYSIS OF STEELS AND ALLOYS. National Bureau of Standards samples Nos. 50a, 132, and 75 were used to study the applicability of the procedure to the determination of tungsten in steels and alloys (Table II). One-gram samples were used and the tungsten was precipitated and separated according to the procedure given for the analysis of tungsten alloys and metals.

ANALYSIS OF ORES. Samples of scheelite and ferberite (obtained through the courtesy of G. E. F. Lundell, National Bureau of Standards, Washington, D. C.), and wolframite (supplied by the Callite Tungsten Corporation, Union City, N. J.) were analyzed by the procedure outlined for the determination of tungsten in ores (Table III). One-gram samples were used.

DISCUSSION OF RESULTS OF ANALYSES

The results obtained in the analyses of the solutions, steels, alloys, and ores show that anti-1,5-di-(*p*-methoxyphenyl)-1-hydroxylamino-3-oximino-4-pentene may be applied to the gravimetric determination of tungsten in these materials. The variation of percentage values between individual samples is well within the range of variation encountered by the use of other acceptable gravimetric methods. With the National Bureau of Standards samples, the values found by this method are within the range of variation of values reported by the various analysts using other methods. The small variations in percentage found by this method are believed to be due to experimental technique rather than any inconsistency in the behavior of the new reagent.

In the analysis of N.B.S. steel 132, which contains approximately equal amounts of tungsten and molybdenum (7 per cent molybdenum and 6 per cent tungsten) it was necessary to determine the amount of molybdenum contaminating the tungsten precipitate and correct for it.

Table I. Standard Tungsten Solutions

Sample	WO_3 Present	WO_3 Found by Cinchonine	WO_3 Found by New Reagent
	Mg.	*Mg.*	*Mg.*
1	9.1	9.1	9.1
2	18.5	18.5	18.5
3	18.0	17.9	18.0
4	18.0	18.1	18.1

Table II. Steels and Alloys

Sample	Material	W Present	W Found	Difference
		%	%	%
1	N.B.S. chrome-tungsten-vanadium steel No. 50a	18.25[a]	18.35	+0.10
2			18.21	−0.04
3			18.27	+0.02
4	N.B.S. molybdenum-tungsten steel No. 132	6.29[a]	6.58	+0.29[b]
5			6.65	+0.36[b]
6			6.73	+0.44[b]
7			6.34	+0.05[c]
8			6.34	+0.05[c]
9	N.B.S. ferro-tungsten No. 75	75.2[a]	75.36	+0.2
10			75.11	−0.1

[a] National Bureau of Standards certificate value.

[b] N.B.S. No. 132 contains 7% (approx.) molybdenum. In samples 4, 5, and 6, no correction was made on ignited tungstic oxide for molybdic oxide as a contaminant.

[c] In samples 7 and 8, molybdenum contaminating oxide was determined colorimetrically and corrections applied. High results of samples 4, 5, and 6 are evidently due to presence of molybdic oxide.

Table III. Ores

Sample	Material	WO_3 Present	WO_3 Found	Difference
		%	%	%
1	Scheelite	59.6[a]	59.53	−0.1
2			59.70	+0.1
3	Ferberite	66.0[a]	66.08	+0.1
4			66.25	+0.3
5			65.97	0.0
6	Wolframite	69.39[b]	69.40	+0.01
7			69.23	−0.16

[a] Tentative values supplied by G. E. F. Lundell. Samples were dried for 18 hours at 105° C. before being analyzed by Dr. Lundell in 1918. They were dried under same conditions for analysis with new tungsten reagent, after each was thoroughly mixed.

[b] Value supplied by Callite Tungsten Corporation on basis of air-dried sample of a commercial wolframite ore.

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Dichromate Determination of Iron, Using the Silver Reductor

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THE method of Walden, Hammett, and Edmonds (*4*) for the determination of iron using the silver reductor and titrating with ceric sulfate, although highly satisfactory, is expensive because of the relatively high cost of the ceric sulfate and its very high equivalent weight. In the titration large quantities of sulfuric acid are required. The dichromate method as presented in this paper overcomes these objections and seems to retain the more desirable features.

In the analysis of ores and alloys of iron the common impurities which may interfere with the reduction and subsequent titration are chromium, manganese, molybdenum, titanium, and vanadium. Molybdenum may be separated from iron by precipitating iron as the hydrous oxide. In the silver reductor, chromium is not reduced below the trivalent state, while manganese and titanium are not reduced at all. Vanadium is reduced to the vanadyl ion which is not oxidized by either cerate or dichromate ions.

Table I. Effect of Added Impurities

(Volume of $FeCl_3$, 25 ml.)

Solution Added		No. of Detns.	Fe Found	Average Deviation
Ml.			*Gram*	*P.p. 1000*
From previous standardization[a]		..	0.1755	...
None		6	0.1756	0.6
25	0.01*M* $K_2Cr_2O_7$	6	0.1757	1.1
25	0.1*M* $MnCl_2$	5	0.1760	2.8
25	0.1*M* $Ti(SO_4)_2$	10	0.1756	0.6
5	0.1*M* $NaVO_3$	2	0.1755	0
10	0.1*M* $NaVO_3$	2	0.1754	−0.6
15	0.1*M* $NaVO_3$	3	0.1754	−0.6
20	0.1*M* $NaVO_3$	2	0.1753	−1.1
40	10 ml. of each	2	0.1754	−0.6

[a] From the standard method of Walden, Hammett, and Edmonds.

The oxidation potential in 1*N* hydronium-ion concentration of the system, VO_4^{---}–VO^{++}, is given as 1.2 volts (*1*), and that of diphenylamine sulfonic acid is 0.83 volt (*2*), which according to Walden, Hammett, and Edmonds (*3*) is sufficiently below the vanadate-vanadyl potential to give precise results. Adjusting the acidity to approximately 1*N* and adding 5 ml. of 85% phosphoric acid, one obtains a condition favorable to the titration of ferrous ion with potassium dichromate even in the presence of vanadium.

MATERIALS

Standard solutions of ceric ammonium sulfate and potassium dichromate were prepared in the usual manner and standardized against pure iron wire. They were also checked against a gravimetrically standardized solution of ferric chloride, using stannous chloride reduction.

A solution of ferric chloride standardized gravimetrically was used in studying the behavior of the dichromate–diphenylamine sulfonic acid titration in the presence of the ions of chromium, manganese, vanadium, and titanium.

The effect of impurities was studied by adding definite quantities of 0.01 molar potassium dichromate, 0.1 molar manganese chloride, 0.1 molar sodium vanadate, and 0.1 molar titanium sulfate. The latter was prepared by fusing 4 grams of titanium dioxide with 80 grams of potassium hydrogen sulfate, dissolving in 55 ml. of concentrated sulfuric acid, and diluting the resulting solution to 500 ml.

The silver reductor was prepared in the manner described by Walden, Hammett, and Edmonds (*4*).

METHOD

Analyses were made with 25-ml. portions of the standard ferric chloride solution by the method of Walden, Hammett, and Edmonds (*4*), titrating with ceric ammonium sulfate. Similar analyses were then made by titrating with potassium dichromate.

A 25-ml. sample of standard ferric chloride solution was pipetted, the acidity was adjusted to 1 molar with hydrochloric acid, and the final volume of 50 ml. was passed through the reductor at a rate of about 30 ml. per minute. The reductor was washed with 150 ml. of 1 molar hydrochloric acid added in small portions. To the reduced solution 5 ml. of 85% phosphoric acid and 5 to 6 drops of 0.25% diphenylamine sulfonic acid were added, and the solution was titrated with potassium dichromate. The results, shown in Table I, deviated from those of the Walden method by only 0.06% error.

Similar analyses were made by introducing in the first series 25-ml. portions of 0.01 molar potassium dichromate, in the second 0.1 molar manganese chloride, and in the third 0.1 molar titanium sulfate. Controls were run on each ion by passing the solution through the reductor in the absence of iron. In each case one drop of 0.1 normal potassium dichromate gave a distinct end point.

It has been shown that reliable results can be obtained in the presence of chromium, manganese, or titanium with acidities ranging between 0.5 and 1.5 molar. Checks were obtained with titration acidities as low as 0.1 molar and as high as 2 molar, but at the extremes of concentration the end points were not sharp. The results shown in Table I indicate that these ions do not interfere with the analysis.

In the presence of high concentrations of vanadium an indistinct end point was obtained, the color change being from light green to gray. Titrations were made in the presence of a wide range of concentrations of vanadyl ion; up to concentrations of 100 mg. of vanadium per 200 ml. of titrating volume a very sharp end point was obtained. The deep violet color was not shown as in the absence of vanadium but there was a distinct color change from light green to deep blue. The results of these runs checked well with the accepted value for iron in the standard ferric chloride solution.

Two samples obtained from the Bureau of Standards were analyzed: iron ore, B. of S. No. 27, and ferrovanadium alloy, B. of S. No. 61. The samples were dissolved according to recommended procedures accompanying the samples. The removal of molybdenum from the alloy was accomplished by twice precipitating the hydrated ferric oxide from ammoniacal solution. In each instance the acidity was adjusted to 1 molar with hydrochloric acid and reduced as before. Results of the analyses are shown in Table II.

Table II. Determination of Iron

B. of S. Sample	No. of Detns.	Fe Found	Fe, B. of S. Certificate	Average Deviation
		%	%	*P.p. 1000*
Iron ore 27	4	69.30	69.26	0.6
Ferrovanadium alloy 61	2	52.83	52.8	...

SUMMARY

Potassium dichromate, using diphenylamine sulfonic acid as indicator, is an oxidizing agent for the determination of iron by the Walden silver reductor method. Manganese, chromium, and titanium do not interfere. Vanadium does not interfere in concentrations of 100 mg. or less in 200 ml. of titrating solution.

A more economical method results from the use of only 1*N* hydrochloric acid instead of the higher concentrations of sulfuric acid required in the ceric sulfate method. The stability, purity, low equivalent weight, and comparatively low cost of potassium dichromate render it a very desirable oxidizing agent in this connection.

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Precise Measurement of Volume in Titrimetric Analysis

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A review of precise measurement of volume in titrimetric analysis is presented. Detailed descriptions of the burets and special techniques used by the author are given. Representative standardizations indicate the reliability to be expected under the prescribed conditions.

ASIDE from the choice of a suitable chemical reaction, or the resultant of a series of successive reactions, on which to base a dependable titrimetric process, one is confronted with the responsibility of performing the physical measurements with sufficient nicety. More specifically, if the highest accuracy is to be attained, the following points must be given serious consideration: (1) influence of temperature change, (2) position of the meniscus, (3) drainage or afterflow, (4) errors of graduation, (5) evaporation, and (6) point of complete reaction.

Except for the last two, these sources of uncertainty can be removed by weighing, instead of measuring by volume, the standard solutions. For this purpose, various forms of "weight" or "weighing burets" have been invented; and a few investigators (*1*, *2*, *15*) have seen fit to take account of the solution of known concentration by weighing the reaction vessel both before and after the titration.

If the loss of water (or other solvent) from standard solutions during long periods of storage be left out of account, it is safe to assume that the evaporation taking place within the time required to effect a volumetric determination is of no significance (*19*). On the contrary, rendering a decision as to the equivalence point looms up as an unquestionably difficult objective in this kind of analysis, and one in which the weight buret, as compared with the volume buret, offers no advantage.

If the volume burets are properly designed, and used with certain precautions, a much higher degree of accuracy may be realized in dealing with a number of the well known volumetric solutions than is commonly supposed. The very fine work of Ponndorf (*26*) may be cited in support of the above contention, and this constitutes by no means the sum total of the evidence (*cf. 11*, *14*, *21*, *48*).

Admittedly, when many titrations have to be made, it is expedient to feed the evaluated solution from a large reservoir directly into the buret. Moreover, in the case of solutions that are sensitive to the oxygen of the air and have to be kept under a nearly constant pressure of an indifferent gas (*4*, *17*), the transfer to a short buret for weighing would probably lead to a lowering of the titer (*28*). Weighing the titration flask is not always feasible: in some experiments the reaction mixture must be heated; it is often desirable to bubble an inactive gas (carbon dioxide, for example) through the test solution (*43*); it is sometimes necessary to introduce an indicator during the latter stages of the procedure. However, Lee (*18*) has developed an ingenious weighing buret, wherein a known solution of titanous sulfate may be obtained by shaking acidulated titanic sulfate with zinc amalgam. But such a device, owing to the high density of the amalgam (about 4 per cent zinc, presumably), would seem to be unduly heavy, besides being restricted to the preparation of only a small quantity of the reducing agent at a time.

The chief limitation to measurement by volume in precise analysis is the lack of experimentally established data regarding the thermal expansion of numerous very useful solutions. This expansiveness is a matter of some importance; hence its further study would seem to be justified.

THERMAL EXPANSION

Thiessen and his collaborators, and also Chappuis, have determined the density of water ("ordinary water-substance") at various temperatures with great exactness. From their data the expansivity may be calculated. Accordingly, Circular 19 of the Bureau of Standards (*5*) enables one to correct the observed volume to what it would be at 20° C.—the standard temperature for volumetric analysis throughout the United States (*24*). The small numbers that are to be added or subtracted have been computed on the assumption that the glass forming the measuring utensils has a coefficient of cubical expansion of 0.000025 per degree Centigrade. (Certain borosilicate glasses, *47*, such as Pyrex, exhibit thermal expansions much smaller than the foregoing.) These corrections apply not only to water but also, practically speaking, to sufficiently dilute aqueous solutions. Furthermore, a supplementary table (*5*, below Table 38) gives the percentage increase in the corrections for water to be applied when standard solutions of four common acids and bases—namely, nitric and sulfuric acids and sodium and potassium hydroxides—are under consideration. This increase is about 5 per cent for the before-named reagents when of 0.1*N* concentration. In like manner, it is but 3 per cent for 0.1*N* hydrochloric acid (*44*)—an almost negligible increment. Yet the higher the normality of the solution the greater must be the augmentation of the temperature corrections for pure water.

As early as 1869, Gerlach (*13*) studied the expansion of aqueous solutions of acids and salts; and in 1877 Casamajor (*6*), utilizing Matthiessen's data for water, attempted to correct the volume of his standard solution for changes of temperature. Some 5 years later, Schulze (*39*) determined the rate of expansion for a good many of the better known volumetric solutions, and these apparently reliable values have been used to a considerable extent. Schloesser (*30*, *31*) and several other authorities (*7*, *9*, *10*, *20*, *25*, *38*) have contributed to the subject in one way or another. Finally, Osaka (*22*) has made available his extensive investigations.

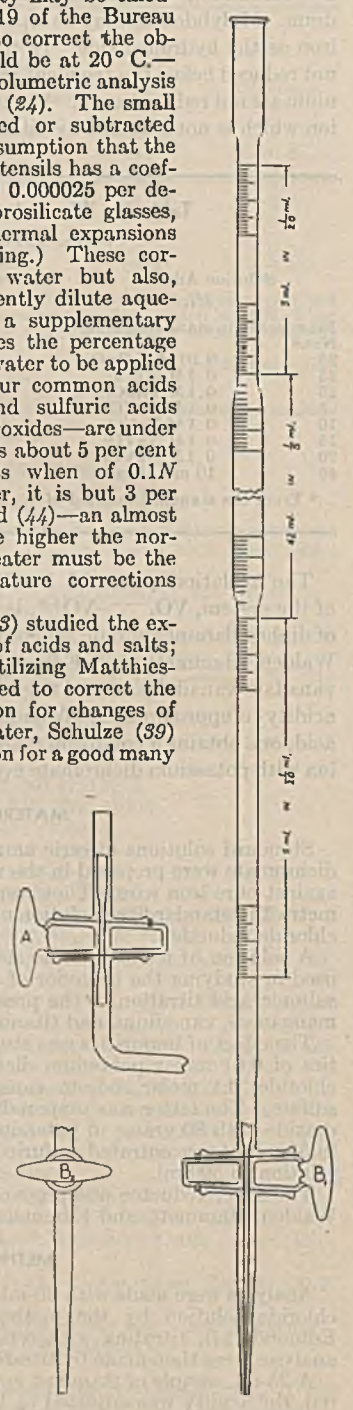

Figure 1

In this connection, it may be desirable to call attention to a point that is apt to be overlooked. When an auxiliary reagent is added to a titrimetric solution, even though it does not enter into the stoichiometric relations, it must needs alter the thermal expansion of the liquid. Many such cases might be cited, but a

few will suffice. Addition of potassium iodide increases the solubility of iodine, acidification of ammonium sulfato-cerate prevents hydrolytic decomposition of the cerium compound, and so on. In all candor, there seems to be a dearth of information concerning the expansivity of the more complex volumetric solutions.

Besides dilatation or contraction, certain other physical properties of an aqueous solution—namely, density, surface tension, and viscosity (*37*)—undergo appreciable change when the concentration with respect to a dissolved substance is markedly increased. As these properties influence delivery, the habitual assumption that the volume measured at a given temperature is equal to that of pure water may not be fully warranted in the case of the complex solutions discussed above.

BURETS

The gratifying results obtained by Getz and the writer (*42*) in standardizing 0.1*N* barium hydroxide (measured from an ordinary buret) against benzoic acid and *p*-nitrobenzoic acid, respectively, led to the expectation that, with a special instrument of comparatively simple construction, it might be possible to attain a very high degree of precision in certain titrimetric analyses.

Two such burets (Figure 1) were procured. They were made by Eimer and Amend, of New York City, in accordance with definite specifications. Each instrument consists essentially of a middle wider portion, graduated in intervals of 0.2 ml., and two narrower portions, one above and one below, both marked in intervals of 0.05 ml. The total capacity is 50 ml., and the entire graduated length is approximately 70 cm. Each stopcock, which is somewhat larger than usual, is composed of a blown (and therefore hollow) plug and a neatly fitting shell. The former turns in the latter with the utmost smoothness. This low-friction bearing and the long handle (more than 5 cm.) permit the operator to release fractions of drops when nearing the end point. These stopcocks do not leak at all.

The following statements and recommendations regarding this type of buret are held to be self-evident. No matter what may be the magnitude of the sample, a reading will be obtained in any titration, provided the required volume does not exceed 50 ml., for the entire interval is graduated—even the shoulders. If, however, the level of the liquid stands between the 3- and the 45-ml. marks, the determination should be taken as tentative only. But, with this datum in hand, it is a simple problem to calculate by means of a proportion what increase in the weight of the sample will give rise to a reading somewhere between the 45- and the 50-ml. graduations, where, on grounds of probability, the best observations are to be had. (The linear value corresponding to 1 ml. is about 34 mm. in the narrower portions of the tube.)

In order to measure volumes somewhat less than 45 ml., a 25-ml. Normax buret was provided. This instrument is fashioned from a straight tube, the smallest division is 0.1 ml., and the distance representing 1 ml. is 16.3 mm.

To make the burets ready for use, a uniform, thin layer of a suitable lubricant is spread between the plug and the shell of the stopcock. With the plug in position B_2, Figure 1, a small amount of carbon tetrachloride is poured into the buret. The key is then turned through an angle of 90°, B_1, and the issuing liquid is caught in a small beaker. The jet being momentarily closed by the index finger, the same portion of the tetrachloride is again introduced into the buret at its top opening; but on no account should the key be turned back to position B_2 at this juncture, for fear that a furrow will be cut in the film of lubricant by the solvent. These operations are repeated until the small tubes have been washed free from visible greasy matter; whereupon the buret is dried thoroughly by aspiration.

The well known "cleaning mixture" (concentrated sulfuric acid to which powdered potassium dichromate has been added in abundance) is then applied, the preceding instructions as to the two positions of the stopcock key (more particularly, the plug capillary) being adhered to in an equally conscientious manner.

In calibrating the burets, the gravimetric method of Peffer and Mulligan (*24*) was followed in a general way. However, great care was taken to discharge the water very slowly—not faster than by rapid dropping from the tip, even during the initial stages of the delivery—and the settings and removal of hanging drops were made with all necessary pains.

Experience has shown that very uniform volumes of pure water may be obtained by slow delivery from a scrupulously clean buret. This observation is in substantial agreement with the words of Osborne and Veazey (*23*): "By limiting the rate of outflow the residue and the afterflow may be made negligibly small."

The results obtained in calibrating the three burets are given in Table I. Judging from duplicate determinations, it is reasonable to hope that, in the case of the special burets (Eimer and Amend 12 and 13, respectively), measurements will agree to within 0.005 ml., and with the 25-ml. buret (Normax 830), to within 0.01 ml.

Table I. Calibration of Burets

(Volumes corrected to 20° C.)

Interval *Ml.*	Delivery *Ml.*	Rounded Value *Ml.*
	E. and A. Buret No. 12	
0– 3	2.996 2.995	2.995[a]
0–25	25.00	25.00[b]
0–45	45.086 45.086	45.085[a]
0–47.5	47.606 47.608	47.605[a]
0–50	50.080 50.082	50.080[a]
	E. and A. Buret No. 13	
0– 2.5	2.517 2.514	2.515[a]
0–25	24.84	24.84[b]
0–45	45.068 45.068	45.070[a]
0–47.5	47.579 47.580	47.580[a]
0–50	50.068 50.067	50.070[a]
	Normax Buret No. 830	
0– 5	4.992 4.994	4.99[b]
0–10	9.995 9.994	9.99[b]
0–15	14.998 14.996	15.00[b]
0–20	19.999 19.999	20.00[b]
0–25	24.997 25.006	25.00[b]

[a] Mean value rounded off to nearest 0.005 ml.
[b] Mean value rounded off to nearest 0.01 ml.

STANDARDIZATION OF SOLUTIONS

By way of forming a fairly satisfactory estimate as to the precision of the physical measurements in really nice volumetric analysis, wherein the titrations are made with volume burets, three familiar titrimetric solutions, of 0.1*N* strength, were standardized in accordance with supposedly accurate methods. The solutions were: (1) potassium permanganate, (2) sodium tetraborate, or borax, and (3) sodium hydroxide.

In the first set of experiments, 0.1*N* solutions of potassium permanganate were evaluated against the certified sodium oxalate of the National Bureau of Standards (Standard Sample 40c), the procedure of Fowler and Bright (*12*) being followed in all its minutiae.

The solutions were prepared by dissolving high-grade crystals of potassium permanganate in the requisite quantity of distilled water, aging for more than a month, and filtering very slowly through glass frit of fine texture under the influence of mild suction.

The clear solution was introduced into the buret by means of the "lift" (Figure 2), an apparatus modeled after a similar contrivance by Osborne and Veazey (*23*). The suction being turned on and properly regulated by obvious manipulations of the stopcocks, *S* and *T*, any desired quantity of the liquid may be pulled up from the storage bottle into a large pipet, *P*, held there at will, and finally allowed to enter the buret. Transferring in this manner leaves the neck of the bottle clean—a condition to be wished for in the case of a permanganate solution.

The temperature of the solution in the buret was taken by inserting a slender thermometer with enclosed scale, just before setting the meniscus upon the 0-ml. graduation; and again at the end of the titration, by running the remaining liquid into a

Table II. Potassium Permanganate against Sodium Oxalate

Solution No.	Date	$Na_2C_2O_4$ Gram	$KMnO_4$ Ml.	Normality
I	June 25	0.311 55	45.325	0.10260
	June 26	0.312 15	45.435	0.10255
	June 27	0.313 15	45.570	0.10257
	July 8	0.310 85	45.225	0.10259
	July 9	0.311 45	45.320	0.10258
			Mean	0.10258
II	May 24	0.316 65	47.420	0.09967
	May 24	0.315 60	47.270	0.09966
	Aug. 5	0.316 85	47.450	0.09967
	Aug. 5	0.316 60	47.415	0.09966
			Mean	0.09966
III	Aug. 16	0.317 05	47.600	0.09940
	Aug. 19	0.317 85	47.725	0.09939
			Mean	0.09940
IV	Jan. 18	0.317 05	47.570	0.09947
	Jan. 20	0.317 90	47.675	0.09951
			Mean	0.09949

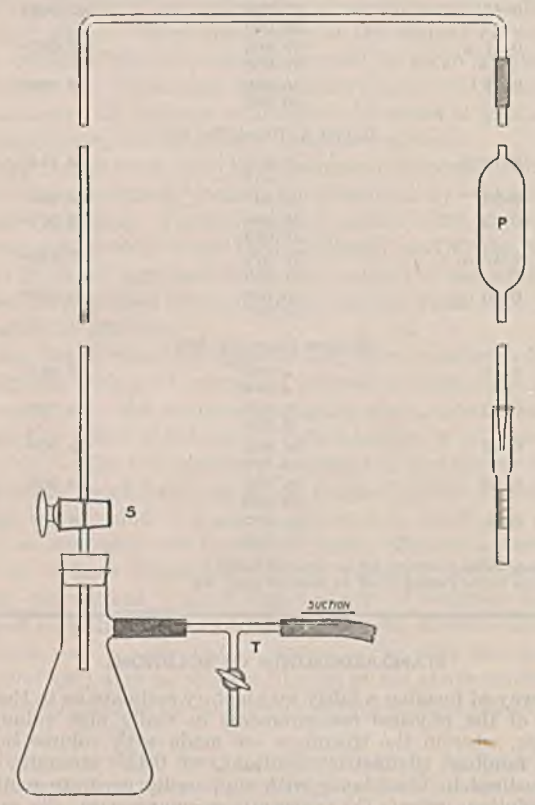

Figure 2. Lift Apparatus

small test tube. The mean of the two values was arbitrarily accepted, and the volume was adjusted on the assumption that the thermal expansion is the same as that of pure water (*22*).

Excellent observations of the meniscus were made by utilizing a device similar to the one proposed by White (*46*), despite the purple color of the solution.

The tiny excess of permanganate unavoidably added was estimated by matching the pink color of the test solution in the usual way. But it is doubtless more accurate to arrive at the correction iodometrically, as suggested by Bray (*3*).

The results obtained with potassium permanganate are set forth in Table II.

In the second set of experiments, the primary standard was constant-boiling hydrochloric acid (*41*), and from this a 0.1*N* acid was obtained. A solution of sodium tetraborate, likewise 0.1*N*, was prepared from recrystallized borax (*16*). For making the standardizations, the borax solution was weighed in a stoppered flask and was then titrated with the hydrochloric acid (in buret 12), methyl red serving as indicator.

A slight lack of uniformity in the results was apparent, owing, presumably, to an end point that is somewhat difficult to judge; and, because of this uncertainty, the tests with borax are not tabulated in the present report. Nevertheless, as the average deviation of a determination was less than 3 parts per 10,000, this part of the work may still be regarded as acceptable.

In the third set of experiments, a 0.1*N* solution of sodium hydroxide was prepared, reasonably free from carbonate, by the method of Cowles (*8*); it was protected against atmospheric carbon dioxide in the usual manner. A second basic solution, in all respects similar to the first, was also employed. The sodium hydroxide was standardized against 0.2*N* hydrochloric acid, which, in its turn, had been obtained by appropriately diluting constant-boiling acid.

For alkaline Solution I (see Table III), a reference color was established by adding 1 drop of methyl red (0.2 per cent alcoholic solution) to 100 ml. of water. For Solution II, 25 ml. of 0.2*N* sodium chloride were diluted to 100 ml., and the before-named quantity of the indicator was introduced. The latter mixture is theoretically correct, but it differs very little from the former in tint.

Twenty-five milliliters of the 0.2*N* hydrochloric acid were measured from the small buret (Normax 830), 25 ml. of water and a single drop of the methyl red were added, and the titration was performed with the 0.1*N* sodium hydroxide (in buret 12), the standard color being matched as well as possible.

The results of this last series of experiments, wherein all the solutions were measured by volume, are given in Table III.

Table III. Sodium Hydroxide against Hydrochloric Acid

Solution No.	Date	0.20066 *N* HCl Ml.	NaOH Ml.	Normality
I	July 19	24.95	47.525	0.10534
	July 19	24.94	47.500	0.10536
			Mean	0.10535
II	Oct. 13	24.98	47.165	0.10628
	Oct. 13	24.98	47.145	0.10632
	Oct. 14	24.98	47.145	0.10632
			Mean	0.10631

CONCLUSIONS

Barring such remarkably accurate results as those attained in iodometry by Washburn (*45*), who used a weight buret of the Ripper type (*27*), the experimental data reported herein compare favorably with many to be found in the scientific literature, which were obtained by weighing, and not by measuring the volume of, the standard solution.

The delivery of a liquid from a graduated tube is at best a somewhat empirical operation, and, naturally, the best measurements may be expected when the physical properties of the solution under consideration are near to those of the liquid (usually water) employed in calibrating the instrument. More especially, the thermal expansion of the titrant should be known, at least approximately. These and many other points have been discussed and elucidated by such prominent metrologists as Schloesser (*29–37*), Osborne and Veazey (*23*), and Stott (*40*), to whose writings the interested reader is referred for further information and advice.

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Ethylbis-2,4-Dinitrophenylacetate, a New pH Indicator

Determination of Saponification Equivalents in Dark-Colored Oils

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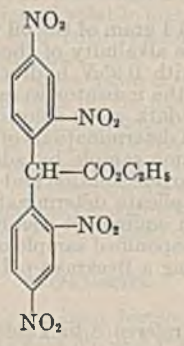
A new acid-base indicator, ethylbis-2,4-dinitrophenylacetate, has been studied and its preparation described. The pH range over which the change from colorless to deep blue occurs is found to be from 7.5 to 9.1 (pK ca. 8.3), making the indicator suitable for most titrations which are ordinarily performed with phenolphthalein. The indicator gives an accurate end point in amber-colored solutions where the phenolphthalein end point is not visible, and it is therefore recommended for use in the determination of acid numbers and saponification equivalents of dark-colored oils.

IN THE course of an investigation on the preparation and properties of substituted phenylmalonic esters, the compound identified by Richter (*6*) as ethylbis-2,4-dinitrophenylacetate was obtained, and the opportunity was taken to study



its indicator properties. Richter noted the color change of this compound from colorless in acid to intense blue in base and accordingly suggested its possible application as an acid-base indicator, but he reported no further studies along this line. The authors' results show not only that the compound may serve as a satisfactory substitute for phenolphthalein in the titration of most weak acids with strong bases, but also that it is specially suited to the titration of orange- and red-colored solutions in which the phenolphthalein end point is not visible. Because of the frequency with which dark-colored oils are encountered in organic analysis and because of the inadequacy of present-day methods of performing titrations with these products (*1–5*), data were obtained to determine the applicability of this indicator for such work. It was found that satisfactory results are obtainable even with extremely dark-colored oils and that the accuracy of the method approaches that of ordinary phenolphthalein titrations.

PREPARATION AND PROPERTIES

The indicator is not difficult to prepare and, while the yield is not good, enough of the compound may be obtained from 0.5 mole of dinitrochlorobenzene to serve for thousands of titrations. The following procedure furnished 19.0 grams (18.1 per cent of theoretical) of recrystallized material sufficiently pure for use as an indicator.

One-half gram-atom (11.5 grams) of sodium was dissolved in 200 ml. of absolute alcohol in a 1-liter three-necked flask fitted with a reflux condenser, motor stirrer, and dropping funnel. The solution was cooled and 0.25 mole of diethyl malonate was added dropwise, with stirring, over a 30-minute period. Stirring was continued for another 10 minutes and a hot solution of 0.5 mole of 2,4-dinitrochlorobenzene in about 200 ml. of absolute alcohol was then added over a 30-minute period. The deep red reaction

mixture was refluxed, with stirring, for 4 hours and allowed to stand overnight. Enough water was then added to the resulting olive-brown solution to bring the volume up to about 1200 ml., after which the solution was acidified with a little concentrated sulfuric acid, stirred for 20 minutes, then allowed to stand for 30 minutes. The water layer was decanted and the residual tar washed twice with water and finally with successive 200-ml. portions of alcohol until a granular black mass was obtained. By repeated washing with hot alcohol an orange solid was finally obtained, which, after recrystallization from benzene, gave pale yellow crystals melting at 150–153.5° C. (uncorrected).

(A small amount of a second compound with indicator properties can be isolated from the alcohol washings; it appears to be the monophenylated ester, ethyl-2,4-dinitrophenylmalonate, which would be expected as an intermediate in the formation of the diphenylated ester. After recrystallization from alcohol this compound melted at 48° C. and dissolved in dilute base to give a deep red color. The change from colorless to red was found to occur over a pH range of ca. 7.2 to 9.0, but the compound is not recommended as a substitute for phenolphthalein because the change is less distinct.)

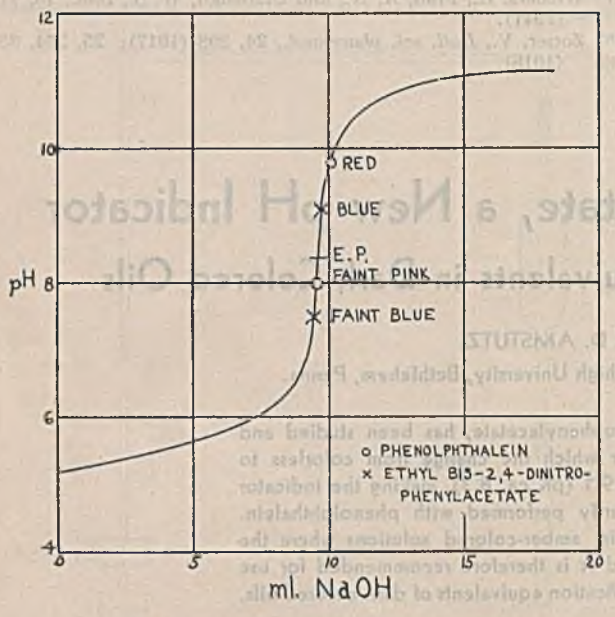


Figure 1. Indicator Ranges in Titration of Potassium Acid Phthalate with Sodium Hydroxide

The exact range over which the color change occurs was determined by titration of a buffered hydrochloric acid solution with sodium hydroxide, using the glass electrode to measure pH. About 5 drops of a saturated solution of the indicator in 50–50 acetone–ethyl alcohol were added to each 100 ml. of solution to be titrated. At pH 7.5 the first blue tinge appeared in the solution, and at pH 9.1 the change to deep blue was complete. (The indicator has been successfully used in the authors' analytical laboratories by students who, by reason of deficiencies in color vision, do not easily recognize the phenolphthalein end point.)

The pK of the indicator is therefore about 8.3. Figure 1 shows the indicator range given by ethylbis-2,4-dinitrophenylacetate compared with that given by phenolphthalein in the titration of potassium acid phthalate with sodium hydroxide. The same reversible color change was observed to occur in anhydrous solvents such as absolute alcohol, dry ether, and dry benzene.

TITRATION OF DARK-COLORED OILS

Since the basic color of ethylbis-2,4-dinitrophenylacetate, instead of being masked in amber-colored solutions as is the case with phenolphthalein, becomes more pronounced as a result of the complementary spectral relations, the end point is easily visible even in titrations involving dark-colored oils. In order to check the accuracy of the end point determined under these conditions, saponification equivalents were determined for samples of pure propyl benzoate and ethyl-3-carbethoxy-2-furanacetate both before and after the addition of an artificial coloring material. For this purpose the addition of a few milliliters of a highly colored neutral caramel solution prepared from pure cane sugar was found to be satisfactory.

Table I. Saponification Equivalents Determined for Colored Oils

(Using ethylbis-2,4-dinitrophenylacetate as indicator)

Oil	Saponification Equivalent Observed			Approximate Color Density, $\frac{i_x}{i_s}$
	Calculated	Uncolored	Colored	
Propyl benzoate	164	165	165	1.85
Propyl benzoate	164	167	166	1.85
Propyl benzoate	164	166	164	1.85
Ethyl-3-carbethoxy-2-furanacetate	113	...	113	1.85
Ethyl-3-carbethoxy-2-furanacetate	113	...	115	1.85
Oil A	...	...	256	1.55
Oil A	...	...	254	1.55
Oil A (electrometric)	...	...	256	1.55
Oil B	...	...	276	60.3
Oil B	...	...	270	60.3

About 0.3 gram of the carefully distilled ester was weighed into a 125-ml. Erlenmeyer flask and the mixture was saponified with 15.00 ml. of a standard alcoholic sodium hydroxide solution according to the procedure of Shriner and Fuson (7). The saponification mixture was titrated almost to neutrality with standard acid, 5 drops of the indicator solution were added, and the end point was determined by adding an excess of acid and back-titrating with standard alkali to the first blue tinge. Several milliliters of aqueous caramel were then added to the alkaline solution and the end point was redetermined in the dark green mixture by adding acid until the amber caramel color just returned. In the dark-colored solutions the end point was approached from the basic side, not from the acid side as in the case of the uncolored solutions.

In Table I the saponification equivalents thus determined for uncolored propyl benzoate and for artificially colored propyl benzoate and ethyl-3-carbethoxy-2-furanacetate are compared with the true values calculated from the molecular weights of the esters. The agreement between the calculated and the observed values is sufficiently good to prove the usefulness of the procedure as an analytical method.

Two heat-bodied linseed oils, one of which (oil B, Table I) was almost opaque when viewed by daylight in thicknesses greater than 5 cm., were studied in order to determine the reproducibility of the results under actual working conditions. Oil A was also subjected to electrometric titration, thus affording an additional check on the accuracy of the results. The following procedure is recommended as a working method.

A sample of 0.2 to 0.4 gram of the oil was saponified as before, and after reducing the alkalinity of the mixture by titrating almost to neutrality with 0.25*N* hydrochloric acid, 5 drops of saturated solution of the indicator were added. More acid was then run in until the dark green color of the indicator just disappeared. Two more determinations of the end point were then carried out on the same sample by adding a few milliliters of standard sodium hydroxide solution and back-titrating with acid. The results of the triplicate determination were then averaged and the saponification equivalent was calculated. The electrometric titration on a saponified sample of oil A was performed in the usual manner, using a Beckman pH meter and a glass electrode.

As can be seen by reference to Table I, the values for oil A obtained by means of the relatively simple indicator method agree with the value obtained from the more elaborate electrometric method, and even the very high color density of oil B does not seriously interfere with the precision of the determination.

The approximate density of the amber color of both the naturally and the artificially colored oils is recorded in the last column of Table I with reference to 0.017*M* potassium dichromate solution arbitrarily assigned a value of unity. The

light absorption was measured by comparison of the various oils with the standard on a Klett-Summerson photoelectric colorimeter without a filter. With the instrument adjusted to give a scale reading, R, of zero for the reference standard, the ratio of the light absorbed by the oil, i_x, to that absorbed by the standard, i_s, was calculated from the equation

$$\log \frac{i_x}{i_s} = 0.002R$$

or

$$\frac{i_x}{i_s} = \text{antilog } 0.002R$$

Thus oil B has a light-absorbing power approximately 60 times that of 0.017M potassium dichromate solution.

ACKNOWLEDGMENT

The authors are indebted to Earl J. Serfass for many helpful suggestions concerning the analytical procedures.

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Pycnometer for Volatile Liquids

Control of Diffusion as an Aid in Precision Pycnometry

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This paper reports the design of a pycnometer which is especially well suited for the determination of density on 5 ml. of volatile liquid with an accuracy of ±0.0001 gram per ml.

FURTHER publication in the field of density determinations might seem unnecessary in view of the large number of devices which have already been described. However, most of these instruments were designed for a special purpose and a simple pycnometer for general use in hydrocarbon analysis, and particularly for obtaining precise densities on materials as volatile as pentane, has not previously been described.

Many general references (*1, 9, 15, 19, 20, 24, 29*) are available on the determination of density, and Irving (*16*) gives a general discussion of the method of floating equilibrium. Unusual methods include measuring the frequency of acoustic vibration (*17*) which is dependent on the density of the surrounding medium. Especially interesting are the balanced column methods (*4, 7, 11, 13*) which eliminate the use of an analytical balance, and which merit further investigation.

The more conventional pycnometers, such as the pipet types (*8, 10, 33*) and the Sprengel-Ostwald type with its modifications (*6, 19, 20, 23, 27*), often involve some kind of closure or cap to prevent rapid vaporization of volatile materials. These caps are in part successful, but are not entirely satisfactory with materials as volatile as pentane and ether.

In 1884 Perkin (*22*) recognized the advantage of having both menisci remote from the ends of the pycnometer arms, so that vaporization would be somewhat hindered. This principle is involved in the design of several flask-type pycnometers (*2, 3, 30, 31*) and many capillary-arm pycnometers (*5, 12, 21, 22, 25, 26*). However, the control of diffusion by the use of an unfilled capillary arm has not been generally recognized and has never, as far as the authors are aware, been discussed on a quantitative basis.

PYCNOMETER

The pycnometer which has been developed (Figure 1) has been in use in these laboratories for about 4 years. It is of Pyrex and consists of a 0.3-, 1-, 3-, or 5-ml. bulb blown in one side arm of a capillary U-tube. About 2 cm. of the upper end of the other side arm are bent over to form a hook for filling the pycnometer by capillary action and for hanging the pycnometer in the balance.

This hook is a self-filling device, previously described by Hennion (*14*). The liquid is first drawn into the pycnometer by capillary action and the pycnometer then fills by siphoning. Parker and Parker (*21*) also used the principle of siphoning to fill their pycnometer. Siphoning has the definite advantage over filling by a suction technique that it reduces the loss of the more volatile components of gasoline.

Both upper side arms of the U-tube are calibrated in scale divisions from 0 to 8.0 with ten intermediate scale divisions between each major scale division. Each major scale division equals 10 mm. The capillary tube should have a 0.6- to 1.0-mm. bore and should not be over 6 mm. in outside diameter. The pycnometer is similar to those of Shedlovsky and Brown (*26*) and Robertson (*25*), which feature two calibrated stems and 25-ml. capacity. The present pycnometer, however, is of much smaller capacity and is easily handled on an analytical balance. It is constructed with a bulb in only one side arm, which allows the pycnometer to be filled easily and avoids loss of volatile products by the slow rate of diffusion through unfilled capillary arms.

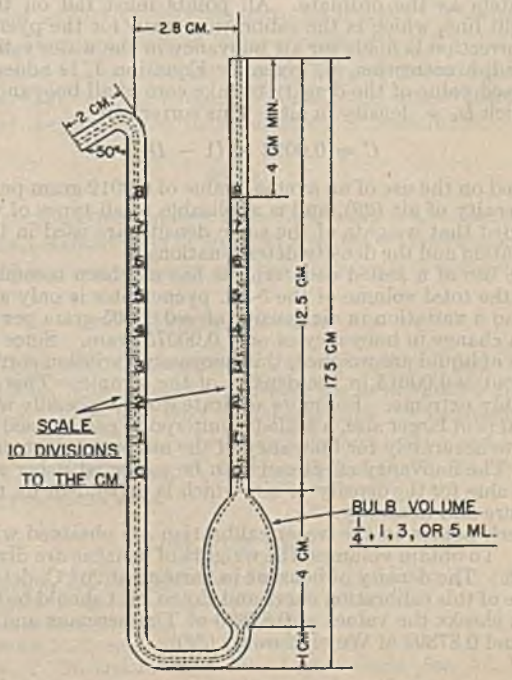

Figure 1. Pycnometer

Capillary bore 0.6 to 1.0 mm., outside diameter 6 mm. maximum, material Pyrex, total weight not to exceed 30 grams

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The data showing the restraining effect of diffusion through a capillary on rate of evaporation, summarized in Figure 2, were obtained using a preliminary design in which both arms of the pycnometer were straight. Recent tests with the pycnometer having one arm bent are similar. Total length referred to is the sum of the unfilled capillary in both arms of the pycnometer and the rate of evaporation is the total rate from both arms of the pycnometer in milliliters per minute. The rates given in Figure 2 are based upon data obtained during July and August in a hot, drafty laboratory. Therefore these rates are probably maximum since heat and drafts on the exposed ends of the pycnometer both increase vaporization. However, these rates are sufficiently accurate to establish the length of the unfilled capillary necessary to avoid volatilization. Under more favorable conditions the evaporation rates are somewhat lower than shown in Figure 2.

It is clear from Figure 2 that if total length of unfilled capillary is over 10 cm., the rate of diffusion is so low that the vapor loss from the pycnometer becomes negligible.

The specification for these pycnometers is 0.6- to 1.0-mm. inside capillary diameter. Since the data were obtained on a pycnometer of 1.0-mm. capillary, the evaporation losses will not be greater than indicated in Figure 2.

CALIBRATION OF PYCNOMETER

The pycnometer calibration is based on the density of water and is checked with a pure hydrocarbon such as benzene. [Benzene may be easily purified by the following procedure: Place 5 gallons of commercial C.P. benzene in a can surrounded with 5 cm. (2 inches) of felt, and place the can in a cold room at about −30° C. (−22° F.), or if this is not available, in a box chilled with dry ice. Allow the benzene to freeze without agitation until only about 1000 cc. of benzene remain uncrystallized. Pour off the impure benzene, melt the crystallized benzene and recrystallize. Each crystallization should take 3 to 5 days. Five or six such slow crystallizations will usually give benzene having a freezing point of 5.51° C. (*31*), and density and refractive index agreeing with the best literature values.]

The volume at 20° C. of water free from air (boiled and cooled without agitation shortly before use) is obtained near the top, bottom, and middle of the scales of the pycnometer. The volume at 20° C. is calculated by dividing the weight of water by 0.99823. A calibration curve is drawn, plotting the sum of the scale divisions on both arms as the abscissa and the volume in milliliters as the ordinate. All points must fall on the same straight line, which is the calibration curve for the pycnometer. No correction is made for air buoyancy in the water calibration. Instead, a correction, C, given by Equation 1, is added to the observed value of the density to take care of all buoyancy errors in which D_0 = density in air. This correction

$$C = 0.0012 \times (1 - D_0) \qquad (1)$$

is based on the use of an average value of 0.0012 gram per ml. for the density of air (*29*), and is applicable to all types of weights, provided that weights of the same density are used in both the calibration and the density determinations.

The use of a sealed counterpoise has not been recommended, since the total volume of the 5-ml. pycnometer is only about 15 ml. and a variation in air density of ±0.00005 gram per ml. will give a change in buoyancy of only 0.00075 gram. Since about 4 grams of liquid are weighed, this buoyancy variation corresponds to about ±0.00015 in the density of the sample. This value is probably extreme. For more accurate work, especially with pycnometers of larger size, a sealed counterpoise can be used to compensate accurately for buoyancy of the air on the pycnometer itself. The buoyancy effect can then be corrected using an accurate value for the density of air, which is dependent on the temperature and humidity.

Check points on the water calibration are obtained with benzene. To obtain volumes, the weights of benzene are divided by 0.8788. The density of benzene in vacuum at 20° C. determined by use of this calibration curve and Equation 1 should be 0.87893, which checks the values of 0.87890 of Timmermans and Martin (*28*) and 0.87895 of Wojciechowski (*32*).

DETERMINATION OF PRECISION DENSITIES

A. Pure Compounds and Mixtures of Medium and Low Volatility. The pycnometer is cleaned with benzene and dried by suction. The final rinse should be with good clean benzene. Acetone is not recommended because it frequently contains nonvolatile residue. If the outside of the pycnometer is dirty or oily, it is rinsed with benzene and dried by gently wiping with cheese cloth or other suitable material. [Static charge introduced by wiping the pycnometer with a dry cloth is apt to cause errors in weighing, especially in cold dry weather. Before hanging pycnometer on wire hook in the balance, observe whether pycnometer exerts attraction for the wire hook by touching the hook with the pycnometer and gently drawing it away. Static charge can usually be nullified by touching the side of the pycnometer lightly with one's fingers. If this procedure will not remove the static charge, the humidity of the balance room should be increased until the difficulty disappears (about 60 per cent relative humidity).] The weight is then obtained within ±0.0001 gram on a good analytical balance, after allowing the pycnometer to come to balance case temperature.

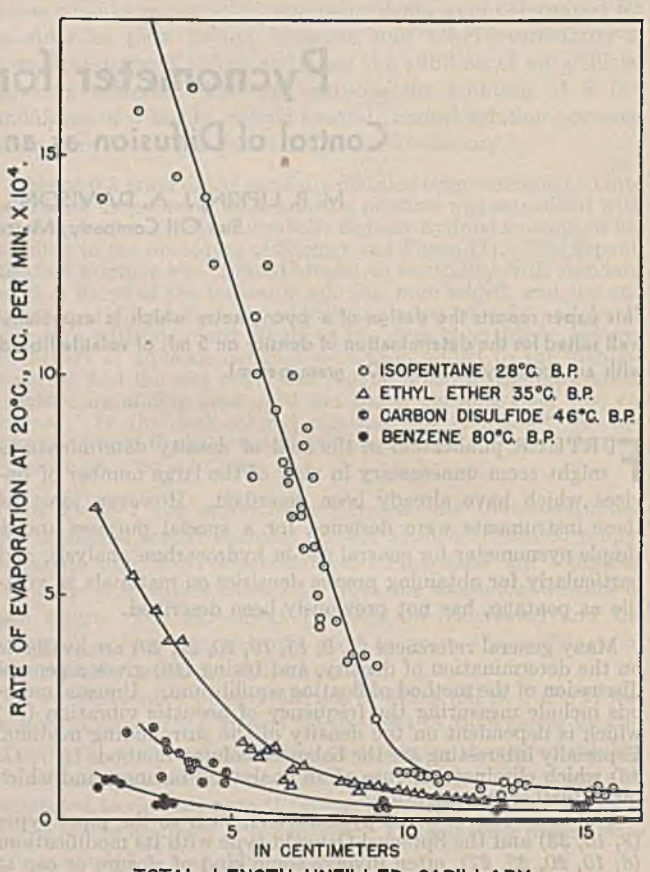

Figure 2. Evaporation Rates

The pycnometer is filled in an upright position by placing the hooked tip in a vial containing the liquid until the liquid level reaches scale mark 4 on the bulb side. Liquid level must be on the scale when the pycnometer contents are at 20° C. (68° F.). In hot weather (100° F.), the contraction to 68° F. on a 3-ml. pycnometer of small bore is approximately 10 cm. If room temperature is below 68° F. or the sample is much below that temperature, due allowance should be made in the filling level. The level of liquid in the vial must be very near the top. Pentane will fill a 3-ml. pycnometer in less than a minute. More viscous samples will fill more slowly. This pycnometer is not recommended for samples more viscous than gas oil of 50 seconds Saybolt viscosity at 100° F. (about 7.4 centistokes).

The pycnometer is removed from the sample bottle, and the hook which had been immersed in the sample is cleaned with a cloth moistened with benzene and wiped dry. The weight is then obtained within ±0.0001 gram.

The pycnometer is placed for 10 to 15 minutes in a vertical position in a glass jar thermostat held at 20.00° ± 0.05° C. and allowed to reach that temperature. A variation of 0.05° C. is equivalent to approximately 0.00004 gram ml. per ml. for gaso-

line hydrocarbon. A more general expression for the temperature coefficient of density of hydrocarbons may be obtained from the correlation of Lipkin and Kurtz (*18*).

The meniscus levels are read on both arms of the instrument within one half of the smallest scale unit without removing the pycnometer from the bath. To avoid undue volatilization losses, the elapsed time between weighing and reading the meniscus levels should not be over 15 minutes. The volume in milliliters corresponding to the sum of the readings is read from the calibration curve:

$$\text{Density in air} = \frac{\text{weight of sample}}{\text{observed volume}} \qquad (2)$$

Equation 1 gives the corrections to be added to the determined density to take care of air buoyancy and to obtain density in vacuum.

B. Highly Volatile Mixtures. This procedure is to be used on highly volatile mixtures because it prevents or reduces change in the compositions of the sample by the selective volatilization of the lower boiling components while the pycnometer is being filled and while it attains balance temperature.

The sample is cooled to ice temperature, charged to the pycnometer, and the pycnometer and contents are allowed to come to temperature, 20° C. The volume is read, the instrument removed from the bath, the outside cleaned and dried, and the pycnometer plus sample weighed. The sample is then flushed from the pycnometer with benzene, and the pycnometer is dried, and weighed empty.

Either procedure may be used on pure compounds of high volatility, since selective loss of one component is not involved. Procedure A is more convenient for samples which are not extremely volatile.

In both procedures there is a time interval between the measurement of weight and the measurement of volume. If volatilization occurs between these measurements in the case of procedure A a high density will result, since weight is measured first. In the case of procedure B, since volume is measured first, low densities will result from such vaporization. Such losses are negligible except in the measurement of very volatile materials such as isopentane in pycnometers of small volume such as 0.3 ml. If vaporization losses are suspected, the density should be determined by both procedures and averaged.

Table I. Deviations in Density Observed Using Pycnometers of Different Sizes[a]

Operator	Material Examined	*d* 20/4	0.32-Ml. Pycnometer	1.2-Ml. Pycnometer	3-Ml. Pycnometer	5-Ml. Pycnometer
1	Isopentane	0.6197	+0.0019	+0.0014	+0.0006	−0.0001
1			+0.0011	+0.0010	±0.0000	−0.0001
2			+0.0084	+0.0025	+0.0005	−0.0002
2			+0.0040	+0.0011	+0.0001	−0.0001
	Av.		+0.0038	+0.0015	+0.0003	−0.0001
1	Ethyl ether	0.7140	+0.0024	+0.0002	−0.0002	−0.0005
1			+0.0022	+0.0006	±0.0000	−0.0001
2			+0.0044	+0.0013	+0.0004	±0.0000
2			+0.0006	+0.0001	−0.0001	+0.0000
	Av.		+0.0024	+0.0005	−0.0002	−0.0001
1	300–400° F. naphtha	0.7975	−0.0003	−0.0005	−0.0001	+0.0001
1			....	−0.0003	−0.0002	−0.0001
2			+0.0004	−0.0007	−0.0003	−0.0003
2			−0.0004	−0.0006	±0.0000	−0.0002
	Av.		−0.0004	−0.0005	−0.0001	−0.0002
1	(50% isopentane) 50% 300–400° F. naphtha	0.7128	−0.0015	+0.0005	−0.0002	±0.0000
1			−0.0022	+0.0003	+0.0002	−0.0001
2			−0.0069	+0.0009	+0.0002	+0.0004
2			+0.0019	+0.0012	+0.0003	±0.0000
	Av.		−0.0031	+0.0007	+0.0002	+0.0001
1	Gas oil 50 sec. Saybolt at 100° F.	0.8304	−0.0032	−0.0005	±0.0000	+0.0001
1			−0.0028	−0.0008	−0.0002	+0.0001
2			−0.0014	−0.0005	−0.0001	±0.0000
			+0.0001	−0.0010	−0.0003	−0.0003
	Av.		−0.0019	−0.0007	−0.0002	−0.0001

[a] Obtained using procedure A and early design of pycnometer with both arms straight. Similar data obtained with bent-arm pycnometer check these data. Earlier tests are given, since they are more complete than recent tests.

PRECISION

Tests of the precision of the pycnometer using procedure A are shown in Table I. (Results obtained with procedure B are of comparable accuracy.) Two operators determined the densities of isopentane, ethyl ether, a naphtha, a mixture of isopentane and a naphtha, and a gas oil. Each operator made duplicate determinations. The data showed that, with the 3- or 5-ml. pycnometer, precision does not depend on the operator, and that the 5-ml. size is precise to ±0.0001, the 3-ml. to ±0.0002, the 1-ml. to ±0.001, and the 0.3-ml. to ±0.002 gram per ml. over the entire viscosity and volatility range between pentane and light gas oil.

The sources of the larger errors in determining densities with the smaller pycnometers are shown in Table II. The effect of the various possible errors on the final determination of the density is given. With the 1-ml. and the 0.3-ml. pycnometers, errors in the scale readings and weighings are important. Errors due to temperature control and air buoyancy are the same with all size pycnometers, as indicated in the table. The 3- and 5-ml. pycnometers are recommended for routine use.

Table II. Errors in Determined Densities of Ethyl Ether Due to Errors of Observation

Volume of Pycnometers[a]	1° C. Error in Temperature	0.5 Mg. Error in Weighing	0.5 mm. Error in Scale Reading
Ml.	*G./ml.*	*G./ml.*	*G./ml.*
0.25	0.0011	0.0020	0.0011
1	0.0011	0.0005	0.0003
3	0.0011	0.0002	0.0001
5	0.0011	0.0001	0.0001

[a] Capillary diameter 1.0 mm.

ACCURACY

The data in column 3, Table I, on the density of the materials were obtained by a skilled operator with the 5-ml. pycnometer, and are believed to be accurate within ±0.0001 gram per ml. If this is true, the data in Table I constitute a test of accuracy as well as of precision. As a further test of absolute accuracy of the authors' method, the density of a carefully purified sample of benzene was determined on a pycnometer calibrated with water. The density at 20° C. was found to be 0.87895. This result checks the literature value of Timmermans (*28*) of 0.87895 corrected from 15° C. and the value of 0.87891 determined by Wojciechowski (*32*) corrected from 25°, using the temperature coefficient of density values determined by Timmermans.

SPEED

Each determination requires 10 to 15 minutes of the operator's time, and an elapsed time of 20 to 30 minutes. With a damped balance, 10 minutes of operator's time per sample is sufficient.

ACKNOWLEDGMENT

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Apparatus for Measuring the Gas Permeability of Film Materials of Low Permeability

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Apparatus is described for measuring the gas permeability of film materials having permeabilities as low as 0.001 cc. (at standard temperature and pressure) per 100 square inches per 24 hours. The low range for previously reported methods (water vapor permeability and balloon fabric permeability) is about 100 cc. per 100 square inches per 24 hours. The apparatus combines simplicity of design, simplicity of manipulation, and high sensitivity in a unit which can be fabricated in most machine shops. The measurements are made under conditions of one atmosphere pressure differential.

THE packaging of some foods, drugs, chemicals, etc., requires flexible films and paper coatings of very low permeability to fixed gases, particularly oxygen. The need for film having a permeability of not more than 0.25 cc. per 645 sq. cm. (100 square inches) per 24 hours has been pointed out by Elder (*3*). This figure is entirely out of the range usually measured for balloon fabrics (*5*), where permeabilities range from 100 cc. per 100 square inches per hour and up. The customary measurements of water vapor permeability (*1*, *2*, *4*) of packaging films are also in a relatively high range—for example, a "good" water vapor barrier will have a permeability figure of 0.1 gram or 125 cc. per 100 square inches per 24 hours. The apparatus described in this paper is designed for measurements in the range 0.001 to 1000 cc. per 100 square inches per 24 hours, and primarily for the measurement of the fixed gases, although with some modification it might be also used for water vapor permeabilities. The purpose of this publication is to make available to others the means which the author has developed for testing packaging films of very low permeability.

The mechanism of gas transmission through solids has not been explained thoroughly. Oswin discusses this briefly (*4*). If the gas passed through small pores or openings in the solid, the situation would be a relatively simple one; the permeability of a film to various gases could be predicted from the known laws of gas flow through orifices. However, such is not the case. It is known that polyvinyl alcohol film is permeable to water vapor but not to oxygen, and Pliofilm is permeable to carbon dioxide but relatively impermeable to oxygen. Perhaps a process of solution of gas in the film on one side, diffusion through the film, and evaporation from the film on the other side takes place in some cases.

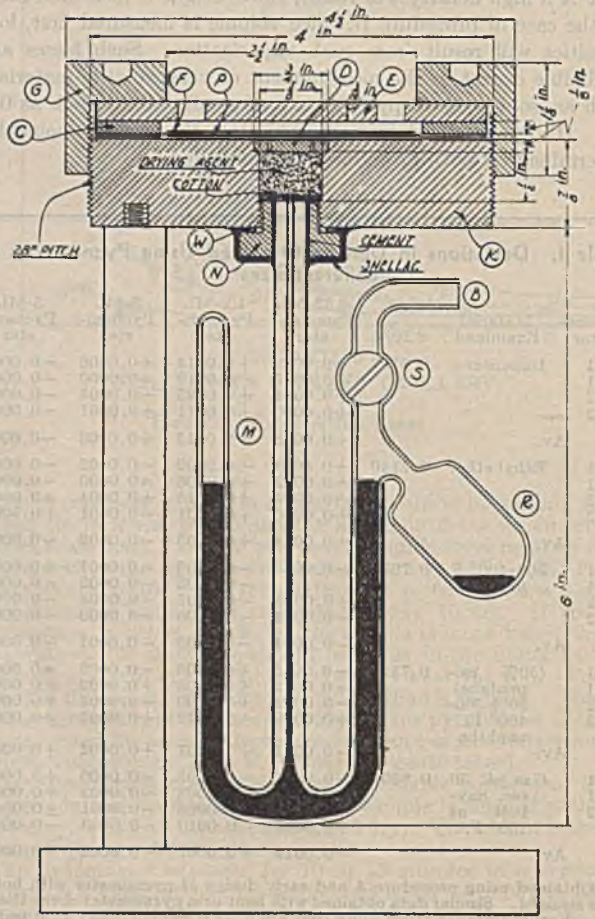


Figure 1. Diagram of Apparatus

The mechanism of gas transmission through film determines to some extent the conditions under which a film should be tested. Two factors are involved: the difference in total pressure on the two sides of the film and the difference in partial pressure of the gas being tested on the two sides of the film. If permeability is due to gas flow through orifices in the film, the difference in total pressure is of primary importance and the difference in partial pressure is of secondary importance. If the permeability is due to solubility of the gas in the film, the difference in total pressure is of no importance, but the difference in partial pressures is important. In the apparatus described in this article, the test is made under the conditions of atmospheric pressure of the gas being tested on one side of the film and zero pressure of all gases on the other side.

The apparatus, shown in Figures 1 and 2, combines simplicity of design, simplicity of manipulation, and high sensitivity in a unit which can be fabricated in most machine shops. Essentially the method involves measuring changes in pressure inside a small evacuated space due to gas passing into that space through the film sample being tested.

CONSTRUCTION OF APPARATUS

The manometer, M, is held in place by the nut, N, to which it is sealed with Cenco Plicene cement or other sealing compounds of good adhesive properties and low vapor pressure. The hole through the nut is bored nearly the same diameter as the glass tube, thereby minimizing the amount of cement necessary for this seal. The nut is screwed up against the thin rubber washer, W, and the entire joint is coated with shellac to ensure a vacuum-tight seal.

Manometer M serves the dual purpose of recording the pressure change and of evacuating the space inside the apparatus. For evacuation, the entire apparatus is tipped so that the mercury runs over into the reservoir, R; then the apparatus is evacuated through tube B with stopcock S in the open position. After evacuation, S is closed and the apparatus is tipped again so that the mercury runs from R into M. In case air leaks through S during a test, the mercury in that arm of the manometer will be depressed, thus serving as an indicator of leakage at this point without spoiling the test. The center arm of M is made of capillary tubing of about 1.5-mm. internal diameter for the purpose of reducing the volume of gas space inside the apparatus and thereby increasing its sensitivity. The rest of the manometer may be made of tubing of any convenient size. The scale used in measuring the height of the mercury is not shown. A piece of millimeter cross-section paper held behind the manometer serves very well for the purpose.

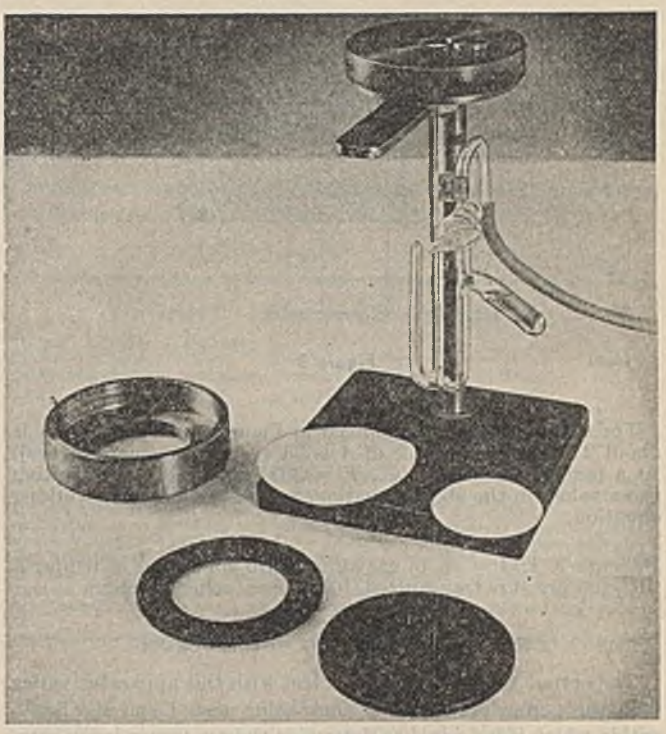
The upper part of the hole in the center of the disk, K, is covered with a small metal disk, D, which has four small holes about 1 mm. in diameter for passing gas into the manometer system. The top surface of the small disk is flush with the top surface of the large disk, K, presenting a continuous smooth surface for mounting the film, F.

The drying agent absorbs all moisture which passes through F, thus eliminating this gas as a source of error in gas transmission measurement. Dehydrite (anhydrous magnesium perchlorate) diluted with a small quantity of Indicating Drierite (anhydrous calcium sulfate) serves admirably. A screen fraction passing 14-mesh and retained on 40-mesh has been found to be a convenient size. In general, any drying agent which will reduce the water vapor pressure inside the apparatus to less than 0.5 mm. of mercury will serve. A small plug of cotton over the top of the manometer tube will prevent particles of the drying agent from falling into the manometer.

MOUNTING SAMPLES

The film sample, F, is mounted on the apparatus as shown in the diagram. A piece of filter paper, P, is placed between the film and K to provide a porous medium for gas leaking through the film to travel to the openings in D and pass through the drying agent into M. The part of F overlapping P and in direct contact with K is sealed to the disk by means of a thin film of heavy stopcock grease having a low vapor pressure. This area of the film is held in place by the rubber gasket, C.

Disk E has two small holes diametrically opposite one another and near the inside circumference of the metal ring, G. When measuring air transmission, these openings are left open so that the space between E and F is filled with air. When it is desired to measure the gas transmission for any other gas, a small rubber stopper with a glass tube is inserted into each of these holes. The space between E and F is then flushed out with gas to be measured by passing the gas in one opening and out the other. It has been shown that the moisture content of fixed gases markedly affects their rate of transmission through some film materials (*4*). This factor may be controlled by conditioning the gas to be tested before it is passed through the apparatus.



Courtesy "Modern Packaging"

Figure 2. Apparatus

In mounting multi-ply film having one or more plies of a porous material—for example, a film laminated between two pieces of paper—it is practically impossible to obtain a vacuum-tight seal using stopcock grease between the paper surface of the sample and K. In mounting such films, use has been made of a plasticized tar as shown in Figure 3. An edge coating extending about 0.6 cm. (0.25 inch) from the circumference is applied to both sides of the disk of film to be tested by dipping in the plasticized tar (Figure 3). The tar-coated disk of material is mounted directly on K without the use of stopcock grease. When cap G is screwed into place, the plasticized tar is spread out by the compressive forces and makes a vacuum seal between F and K. Carbowax 1500 is a suitable plasticizer for the tar when used at the level of about 5 per cent.

CALCULATIONS

Gas transmission measurements are conveniently recorded as cubic centimeters of gas at standard conditions of temperature and pressure transmitted per 100 square inches of surface per 24 hours. Such a figure is given by the following expression:

$$\frac{P}{760} \times V \times \frac{273}{T} \times \frac{100}{A} \times \frac{24}{\text{hours}}$$

where P = the absolute pressure inside the apparatus as measured on the manometer in millimeters
V = total volume of the inside of the apparatus in cubic centimeters
T = temperature in degrees absolute
A = surface area of test sample (area of filter paper P in square inches)
hours = time of test

The value of V may be calculated with sufficient accuracy (within 5 per cent) from the known dimensions of the apparatus, making a correction for the volume of the drying agent used.

It is equal to the sum of the volume of the holes in D, the volume of the circular opening containing the drying agent minus the volume of the drying agent, and the volume of the capillary bore of the center stem of the manometer down to the mercury level in this stem. The volume of the pore space in P amounts to about 0.05 cc. and the volume change due to a mercury level change of 20 mm. in the center stem of the manometer amounts to about 0.04 cc. These errors in volume are neglected.

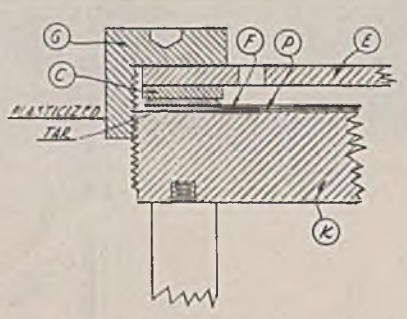


Figure 3

For the apparatus diagrammed in Figure 1, the value of V is about 2 cc., and the value of A is 23 sq. cm. (5 square inches). At a temperature of 30° C., T = 303° absolute. Substituting these values in the above equation, we get the following working equation:

P/hours × 1.14 = cc. of gas at standard conditions of temperature and pressure transmitted/100 square inches/24 hours

SENSITIVITY, ACCURACY, AND PRECISION

The actual time required for a test with this apparatus varies with the permeability of the film being tested and the limits within which it is desired to determine the gas transmission rate.

For example, it might be desired to know whether a film sample has a transmission rate greater or less than 0.25 cc. per 100 square inches per 24 hours. Using the above equation and constants for the apparatus herein described, this transmission rate will produce a pressure change in the manometer of 0.5 mm. in about 2.5 hours: 0.5-mm. change is easily read on the manometer; therefore, the failure of the manometer to change 0.5 mm. in 2.5 hours' time is sufficient evidence that the gas transmission rate is less than 0.25 cc. per 100 square inches per 24 hours. Should the time of the test be extended five times as long, to 12.5 hours, the failure of the manometer to change 0.5 mm. is sufficient evidence that the gas transmission rate is less than 0.05 (0.25/5) cc. per 100 square inches per 24 hours. Further extending the time of the test thus further reduces the figure for the maximum transmission rate of the sample.

With films of high permeability, the mercury in the manometer will drop several millimeters in an hour's time. With such films, therefore, the test need not be extended more than a few hours.

Occasionally film samples contain material, such as plasticizers, which have appreciable vapor pressures. Such materials will vaporize into the apparatus and depress the mercury in the manometer. This may be mistaken for gas transmission through the film, but may be distinguished from gas transmission by the fact that the pressure will reach a constant value, the rate of change of pressure decreasing as this value is approached. It is therefore necessary to make readings of the manometer at intervals during the test and calculate the rate of manometer change for each interval. If the rate of change is constant, it is due to gas transmission through the film, but if it is a decreasing rate of change, the change is due at least partly to vaporization of some volatile material from the film.

The required accuracy is not very great for measurements of permeability of film packaging materials where values might vary a millionfold among various materials and a thousandfold among various samples of the same material. It is estimated that measurements with this apparatus are accurate to within 15 per cent. The temperature, time, and manometer reading may be determined within a few per cent. The principal factors affecting the accuracy are the volume of the apparatus and the area of the test sample. It is estimated that these are easily determined in the manner described within 10 per cent accuracy. The precision or reproducibility of measurements on duplicate samples of the same material is generally about 10 per cent.

EXAMPLES

The following examples will serve to typify the data which may be obtained with this apparatus:

1. Air transmission for an impermeable sample (a sample of laminated glassine at 60 to 80 per cent relative humidity).

In a period of 36 hours, no change was observed in the manometer. Assuming that a minimum change of 0.5 mm. can be detected on the manometer, the rate of change of the manometer was not greater than 0.5/36 or 0.0139 mm. per hour. The gas transmission was, therefore, not greater than 0.0139 × 1.14, or 0.016 cc. per 100 square inches per 24 hours.

2. Air transmission for an impermeable sample containing a volatile plasticizer (a polyvinyl alcohol coating at low humidity).

The manometer changes during the indicated time intervals were as follows:

Time Interval *Hours*	Manometer Change *Mm.*	Rate of Change *Mm./hour*
0–1	2	2.0
1–2	1.5	1.5
2–4	2	1.0
4–10	3.5	0.58
10–40	0.5	0.017
	Total 9.5	

It is evident from the above data that a material having a vapor pressure of about 9.5 mm. is evaporating from the sample and that equilibrium is nearly established after 10 hours. The rate of change of the manometer during the last 30 hours of the test was 0.017 mm. per hour. Therefore, the gas transmission rate for the sample was not more than 0.017 × 1.14 or 0.019 cc. per 100 square inches per 24 hours.

3. Air transmission for a permeable sample (a thin coating of polyvinyl alcohol).

The manometer changes during the indicated time intervals were as follows:

Time Interval *Hours*	Manometer Change *Mm.*	Rate of Change *Mm./hour*
0–1	1.5	1.5
1–2	1.5	1.5
2–4	3.0	1.5
4–10	9.5	1.58
10–18	11.5	1.45
Total 18	27.0	Av. 1.5

The constancy of the rate of change figures indicates that this sample is permeable to air. The rate of gas transmission is 1.5 × 1.14, or 1.71 cc. per 100 square inches per 24 hours.

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Design of Large-Size Laboratory Extraction Glass Apparatus

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A SIMPLE large-capacity extraction apparatus has been described by Smallwood (*2*).

The frequent need for extraction apparatus of larger capacity than the conventional Soxhlet extractors for research as well as for small pilot production justifies a description of the improved all-glass apparatus which has been in satisfactory operation in this laboratory for a number of months.

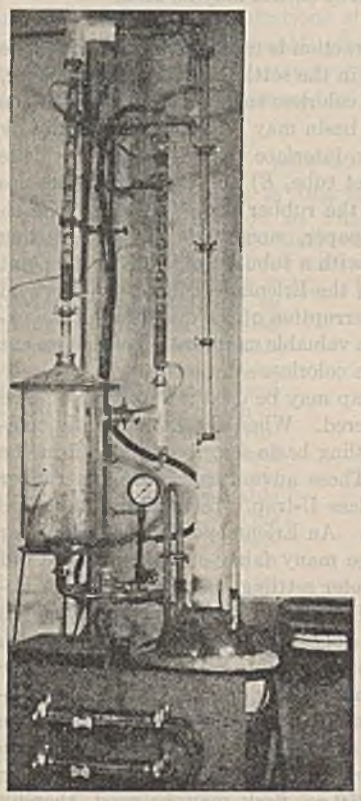

Figure 1

The apparatus is shown in Figure 1, after removal of the insulation. It is designed for operation at either ordinary or reduced pressures and is equipped to permit the collection of all engineering data required for pilot work. It is made of standard Pyrex parts with Ace spherical joints, size 35/25. The boiler is a three-neck 12-liter flask installed in a Glas-Col heater provided with proper input control (rheostat and ammeter) and pyrometer. The extractor has a total capacity of 8 liters. In order to obtain a sufficient speed of extraction the hot solvent vapor is fed at the top of the 600-mm. Allihn condenser, instead of the bottom, as in the conventional Soxhlet. Experiment has shown that flooding occurs with the conventional design at a distillation rate of 3 liters per hour at ordinary pressure and 1.5 liters per hour at 74.1 mm. hg. (27-inch) vacuum in the presence of alcohol vapor, with a condensing area of 640 sq. cm. cooled with brine, whereas the present design permits an extraction of 5.5 liters per hour at ordinary pressure and over 4 liters per hour in vacuum with a condensing area of only 350 sq. cm.

When used under vacuum, excessive evaporation of the solvent in the extractor is prevented by a cack-condenser inserted in the vacuum line (350-sq. cm. Allihn Pyrex vertical condenser) at the level of the side connection of the 8-liter bell jar forming the extractor.

Inasmuch as the sudden emptying of the large extractor into the hot boiler could be dangerous, particularly with very volatile solvents, the return U-tube is provided with a glass stopcock which permits either controlled periodical or continuous circulation of the solvent. A sampling outlet is provided at the bottom of the extractor.

The condensers can be cooled with either water or brine, and the piping circuit includes the necessary valves and measuring devices for determination of the heat balance.

Table I indicates the performance of the apparatus in two different experiments.

The volatility involved in calculating the over-all theoretical plate number has been calculated from the recent data of Beebe and co-workers (*1*): For $x_f = 24.10$ and $x_0 = 56.75$ the overhead temperature was always lower than 39.3° C., the difference being probably accounted for by the presence of noncondensable gas in the vapor phase, which is difficult to avoid in apparatus of relatively large size. Despite the length of the vapor line, the apparatus appears well suited for use with mixed or diluted solvents.

Some determinations of the approximate heat balance have been made and one typical result is reported in Table II.

Table I. Performance of Apparatus

Head Temperature ° C.	Boiler Temperature ° C.	Extract Temperature ° C.	Absolute Pressure *Mm. Hg*	Overhead *Mole %*	Bottom *Mole %*	Plate No.[a]
32	36	27	109	73.0	23.5	1.4
29	35	26	100	62.8	23.5	1.1

[a] Over-all theoretical plate number. Although a certain reflux takes place from the top of the vapor line downward, no liquid is ever carried back to the still pot when the heat input is correctly adjusted. Therefore the phases in equilibrium considered for the calculation of the "over-all theoretical plate number" were the still pot liquid composition and the distillate composition, respectively. No flow measurement is involved in this calculation. However, the rate of distillation can be determined by measuring the time required to fill the extractor whose capacity is known; this operation requires shutting off the stopcock on the return U-tube for only a short time.

The data reported show the excellent performance of the apparatus. They demonstrate the possibility of obtaining a rate of extraction much faster than hitherto reported with large all-glass laboratory apparatus, while at the same time maintaining a satisfactory and economical heat balance so that the extraction can be carried out under conditions more closely resembling those of plant operation.

Table II. Heat Balance

Heat Input from Distillate	Heat Output (to Brine)
A. Heat of condensation Rate of distillation, ml. per min., 80 Distillate, sp. gr., 0.840 Ethanol, mole %, 62.8 Water, mole %, 37.3 Sp. heat of ethanol, 204.26 Sp. heat of water, 579.3 A = 23,103.96 calories per min.	Rate of brine flow, liters per min., 5.6 Brine, sp. gr., 1.20 Brine, sp. heat, 0.7 Input temperature, ° C., 8.0 Temperature increase, ° C., 8.1 Weight, gal. of water, lb., 8.33 Heat output, 81.4 B.t.u. or 20,462.4 calories per min.
B. Heat of of cooling of distillate Temperature decrease, ° C., 29–26° = 3° Sp. heat of 62.8 mole % of ethanol at 23° C., calories per gram, 72.0 B = 145.15 calories per min.	
Total heat input, 23,249.11 calories per min.	Balance[a], 2787 calories per min. or 11.9%

[a] "Balance" represents summed effect of heat losses from the still pot and the vapor line, the thermometers, various experimental uncertainties, and some simplifying assumptions made in calculation of the quantities involved.

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PRESENTED before the Division of Industrial and Engineering Chemistry at the 105th Meeting of the AMERICAN CHEMICAL SOCIETY, Detroit, Mich.

A Continuous Liquid-Liquid Extractor

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RECENTLY Kieselbach (*1*) introduced a new principle into the field of liquid-liquid extractors for use with mixtures which tend to emulsify. His extractor, using air agitation and a settling chamber for the separation of any emulsion formed, made possible the rapid extraction of solutions formerly taking as long as several weeks. However, Kieselbach's extractor has a number of disadvantages, chief among which are the facts that a separate extractor is necessary for each desired volume of solution to be extracted and that very stable emulsions do not separate in the narrow settling chamber.

The apparatus herein described was an attempt to increase the utility of Kieselbach's extractor for use with strongly emulsifying sulfite waste liquor reaction mixtures varying in volume from 250 cc. to several gallons. The extractor unit is actually an adapter to be used with standard-taper glass bottles or flasks. It is made from readily available stock glassware and requires a minimum of glass blowing.

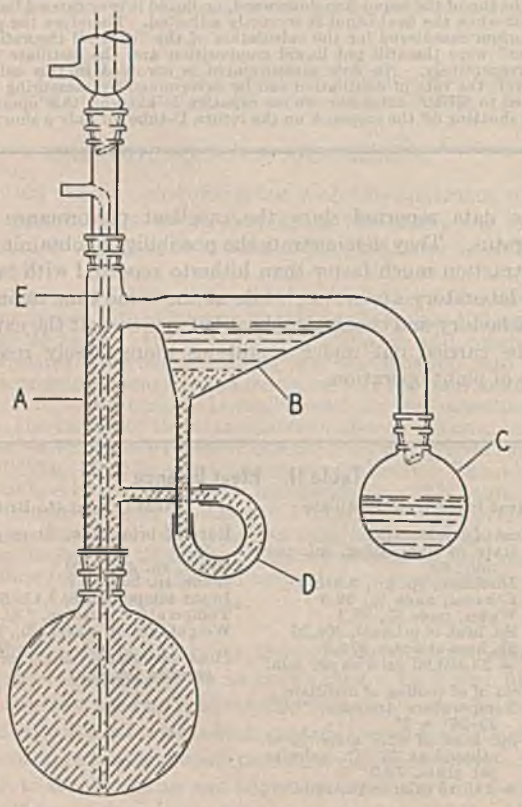

Figure 1

The operation of the complete extractor assembly drawn to scale in Figure 1 is identical to Kieselbach's. The extractor unit was used successfully for extracting solutions contained in vessels ranging from 250-cc. flasks to 12-liter bottles.

The mixing chamber, *A*, was made by sealing together two standard-taper glass joints. The upper joint in this case was 24/40, although any size compatible with other equipment is satisfactory. The lower joint was 29/42; because of the possibility of constriction, a smaller standard-taper joint should not be used. Both standard joints were connected to 28-mm. outside diameter tubing. A 500-cc. Erlenmeyer flask served as the settling chamber, *B*, and was connected to the mixing chamber by a short piece (30 mm.) of 25-mm. outside diameter tubing. A 24/40 standard-taper joint was used for the connection to the boiling flask, *C*. Tubing of at least 10-mm. outside diameter should be used for the glass part of the trap, *D;* otherwise, the passage of very slowly breaking emulsions is impeded. The trap itself was made of a short length of fairly thick-walled neoprene tubing. The inner tube, *E*, was made by sealing a piece of 8-mm. outside diameter glass tubing to a stock gas inlet adapter. The length of the tube depended upon the size of the flask containing the material to be extracted. If a number of gas inlet adapters are not available, a 10/30 standard joint may be sealed to the stock adapter and a number of 8-mm. tubes with standard 10/30 joints of lengths to fit various flasks or bottles may be used.

The completion of the extraction is usually determined by the change in color taking place in the settling chamber. However, in the case of extractions of colorless substances, samples of the solvent layer in the settling basin may be taken periodically by lowering the solvent solution interface (by withdrawing a little solution through the gas inlet tube, *E*) and the appropriate use of several screw clamps on the rubber trap. (Richard Kieselbach, after reviewing the paper, suggested that the settling chamber might be provided with a tubulature, close to the point which used to be the neck of the Erlenmeyer flask, which would permit sampling without interruption of the operation of the extractor. This should prove a valuable modification of the present apparatus when dealing with colorless solutions.)

A glass T in the rubber trap may be used if a number of such solutions are to be encountered. When the extraction is complete, the solvent in the settling basin may be drawn off in the manner described above. These advantages make the rubber trap preferable to the all-glass U-trap. In addition, the glass blowing is greatly simplified. An Erlenmeyer flask was used for the settling chamber because many fairly stable emulsions did not break in the small-diameter settling basin of the earlier apparatus. The shape of the Erlenmeyer flask is admirably suited for this purpose because the slopes of its base and sides (when in the position shown in Figure 1) facilitate rapid separation of the two liquid phases. Furthermore, this design is relatively compact. For maximum applicability, the seals at both ends of the Erlenmeyer flask should be at the same level. The size of the flask depends upon the nature of the solutions encountered. For fast-breaking emulsions a 250-cc. flask may be used, thereby holding up less solvent in the settling chamber. If a large mixing chamber is used with a correspondingly increased air stream, a larger settling chamber should be used. The specifications of the entire apparatus are flexible.

When extracting materials subject to oxidation by air a stream of inert gas should be used for agitation. Furthermore, when extracting gas-saturated solutions (such as bicarbonate or bisulfite solutions), carbon dioxide or sulfur dioxide, respectively, may be used advantageously as the agitating gas. In extractions of large volumes of solutions with corresponding increases in time, the loss of solvent due to extrainment in the exit gases may become appreciable, and more solvent may have to be added to the boiling flask. In this case advantage may be taken of a two-necked boiling flask. A long efficient reflux condenser should always be used. An active carbon trap is useful for recovering larger amounts of solvents.

A large apparatus, using semiball joints, for use with 22-, 50-, and 72-liter flasks was fabricated according to specifications by the Scientific Glass Apparatus Co., Bloomfield, N. J.

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Determination of Small Amounts of Acrylonitrile in Air

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THE recent widespread use of acrylonitrile in the manufacture of one type of synthetic rubber has made it imperative that a quantitative method for its determination in air be developed. The toxicity experiments conducted by the U. S. Public Health Service (*1*) indicate that the maximum allowable limit for acrylonitrile is in the neighborhood of 20 p.p.m. Consequently, an analytical method must be accurate to at least that concentration. As far as is known no analytical method has yet been reported. The Raleigh-Jeans gas interferometer has been used for concentrations above 90 p.p.m. Below this figure the results are questionable (*2*). In addition, the gas interferometer is not very satisfactory for field use where mixtures of vapors are likely to be encountered.

METHOD

The method developed in this laboratory depends upon a modified Kjeldahl reaction in which the absorbing solution containing the acrylonitrile is made strongly alkaline with sodium hydroxide and then oxidized with hydrogen peroxide. Upon refluxing, the acrylonitrile is converted quantitatively to ammonia, which is distilled over and collected in a standard acid solution. The amount of ammonia evolved is determined by titration of the excess acid. The acrylonitrile vapors are absorbed in cold concentrated sulfuric acid contained in a suitable absorption trap. This method is limited by the fact that there can be present in the contaminated air no nitrogen-bearing compounds other than acrylonitrile.

PROCEDURE

SAMPLING. Two absorption traps (Figure 1) are filled to a depth of about 2.5 cm. (1 inch) with glass beads, and 2 ml. of concentrated sulfuric acid are added to each trap. The traps are connected in series and put in a water-ice bath. The air sample is drawn through at a maximum rate of 0.4 liter per minute. The rate of sampling is accurately measured by means of a rotameter. The volume of air sampled should be such that the total sample consists of approximately 6 mg. of acrylonitrile. The sample is then washed into the reflux flask (Figure 2), and 0.2 gram of copper acetate is added as an inhibitor to prevent polymerization.

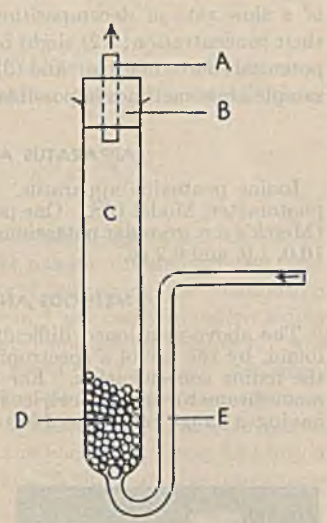

Figure 1. Absorption Trap

A. Glass outlet tube
B. One-hole rubber stopper
C. 0.25-inch test tube
D. Glass beads
E. Glass inlet tube

ANALYSIS. Twenty-five milliliters of 0.025*N* sulfuric acid are pipetted into the titration beaker and diluted with distilled water until the bubbler is at least 1.25 cm. (0.5 inch) below the surface. The reflux flask containing the sample is put into place and the system is completely closed. The acid sample is made alkaline by adding 50 ml. of 50 per cent sodium hydroxide to the closed system by means of the separatory funnel. The residual sodium hydroxide in the reflux condenser is washed down with 10 ml. of distilled water, and 20 ml. of 30 per cent hydrogen peroxide are then added slowly from the separatory funnel. When the addition is completed, the sample is refluxed gently for 30 minutes. At the end of the reflux time, the water is drained from the reflux condenser and approximately one half of the sample is distilled over. The second condenser is then washed down with distilled water, and the excess sulfuric acid is titrated with 0.01*N* sodium hydroxide, using methyl red as the indicator.

Table I. Accuracy of Method over Wide Range of Concentrations

Theoretical Concentration	Sampling Rate	Sampling Time	Yield		
P.p.m.	*L./min.*	*Min.*	*Mg.*	*P.p.m.*	*%*
400	0.4	20	6.54	394	98.5
200	0.4	35	5.51	189	94.6
100	0.4	75	6.12	97.9	97.9
50	0.4	150	6.29	50.6	101.2
25	0.4	180	4.50	30.0	120.0

The concentration of acrylonitrile is calculated as follows:

$$\text{P.p.m.} = (25.00N_A - N_BV_B) \times \frac{1}{V_S} \times C$$

when N_A = normality of H_2SO_4
V_B = ml. of NaOH used in titration
V_S = volume of air sampled, liters
N_B = normality of NaOH used in titration
C = 22,400 corrected to sampling pressure and temperature

DISCUSSION

The analysis is based upon Radziszewski's reaction (*3*). The mechanism is as follows:

$$RCN \xrightarrow{H_2O_2} R{-}C(=O){-}HN_2 \xrightarrow[\Delta]{NaOH} NH_3 + R{-}C(=O){-}ONa$$

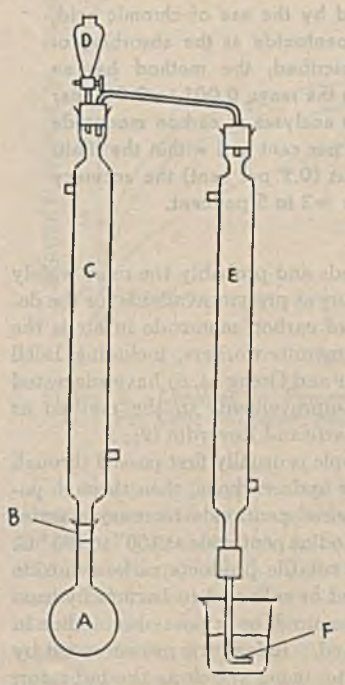

Figure 2. Apparatus for Determination of Acrylonitrile

A. 200-cc. balloon flask
B. Ground-glass joint
C. Reflux condenser
D. Separatory funnel
E. No. 2 condenser
F. Sintered-glass bubbler

The use of hydrogen peroxide reduces the reflux time from 4 hours to 0.5 hour, and also drives the reaction to completion. Low yields due to polymerization of the acrylonitrile are prevented by the addition of copper acetate as an inhibitor, and by limitation of the size of the sample. A sample containing approximately 6 mg. of acrylonitrile proved to be most satisfactory because it is dilute enough to prevent polymerization and yet large enough to give an accurate analysis. The maximum sampling rate of 0.4 liter per minute is very critical for the type of absorption equipment described in this article. A higher rate will result in loss of sample.

Table I contains the results of the analyses of known concentrations of acrylonitrile vapors, prepared in a gas chamber of 1000 liters' capacity by the introduction of measured amounts of liquid acrylonitrile. The various concentrations were chosen to indicate the accuracy of the method over a wide range.

Over the entire range of concentrations the only variable is the time of sampling. The large relative error in the analysis of the 25 p.p.m. samples is misleading, since the actual error is only 5 p.p.m. The principal reason for the error in the results of the low concentrations is the difficulty of measuring the exceedingly small amounts of liquid acrylonitrile required in making up these concentrations in the 1000-liter gas chamber available.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the cooperation of V. Migrdichian of the American Cyanamid Company for his suggestions as to the sampling medium, and of W. P. Tyler, T. R. Steadman, and J. C. McCool of the B. F. Goodrich Company in the development of the analysis.

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Spectrophotometric Determination of Iodine Liberated in the Oxidation of Carbon Monoxide by Iodine Pentoxide

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A spectrophotometric method for measuring the iodine liberated in the oxidation of carbon monoxide by iodine pentoxide is described. The iodine concentration was measured at 350 millimicrons against a water blank, using a Coleman photoelectric spectrophotometer. Results in terms of carbon monoxide were calculated by the use of a formula derived from the calibration curve and the chemical reactions involved. The method is sensitive, convenient (requiring the preparation of only one reagent, 1 per cent potassium iodide), and reliable for concentrations of carbon monoxide as low as 0.005 to 0.001 per cent. The range of applicability is 0.001 to approximately 0.2 per cent. Interfering substances (gasoline vapor, water, etc.) are effectively removed by the use of chromic acid, silica gel, and phosphorus pentoxide as the absorbing or "scouring" agents. As described, the method has an accuracy of ±10 per cent in the range 0.001 to 0.010 per cent carbon monoxide. For analyses of carbon monoxide concentrations above 0.010 per cent and within the limits of usefulness of the apparatus (0.2 per cent) the accuracy of the method is increased to ±3 to 5 per cent.

ONE of the standard methods and probably the most widely used quantitative procedure at present available for the determination of small amounts of carbon monoxide in air is the iodine pentoxide method. Numerous workers, including Edell (*1*), Sendroy (*3*), and Vandaveer and Gregg (*4*, *5*) have suggested modifications or have added improvements to the method as originally introduced by de la Harfe and Reverdin (*2*).

In these methods the gas sample is usually first passed through chromic acid to remove volatile hydrocarbons, then through potassium hydroxide and phosphorus pentoxide to remove water vapor, and finally over dry solid iodine pentoxide at 150° to 160° C. to produce quantitatively the volatile products carbon dioxide and iodine. These are absorbed or collected in barium hydroxide in case carbon dioxide is measured, or in potassium iodide in case the iodine is to be determined. Iodometric measurement by titration with sodium thiosulfate, using starch as the indicator, has been generally adopted as the method of choice.

However, the necessity for frequent determinations in this laboratory of low (0.005 to 0.001 per cent) carbon monoxide concentrations disclosed several difficulties and disadvantages which made desirable some other means of measuring the iodine liberated. The following may be mentioned specifically: (1) the very dilute (0.001*N*) sodium thiosulfate solutions used, because of a slow rate of decomposition, require periodic checks upon their concentration; (2) slight overtitration of the end point is a potential source of error; and (3) duplicate titrations on the same sample are sometimes impossible.

APPARATUS AND REAGENTS

Iodine pentoxide apparatus. Coleman photoelectric spectrophotometer, Model 10S. One per cent potassium iodide solution (Merck's C.P. granular potassium iodide is satisfactory). Pipets, 10.0, 1.0, and 0.2 cc.

METHODS AND PROCEDURE

The above-mentioned difficulties could be eliminated, it was found, by the use of a spectrophotometric method of measuring the iodine concentration. For this purpose a Coleman double monochromator photoelectric spectrophotometer (Model 10S) having a range of 350 to 1000 millimicrons was used. Determinations of the spectral transmittance of iodine in 1 per cent potassium iodide, the calibration curve relating concentrations to logarithm of the percentage transmittance, as well as all measurements of iodine concentration in blanks and unknown samples were performed with this instrument.

Figure 1. Absorption Tube

The spectrophotometric method of measuring the amount of iodine in a 1 per cent potassium iodide solution requires no chemical reaction of the iodine but is dependent only upon the absorption of light by the iodine in solution. For the range of iodine concentrations used this absorption has been observed to follow Beer's law, in that the relationship between the logarithm of the percentage transmittance and the con-

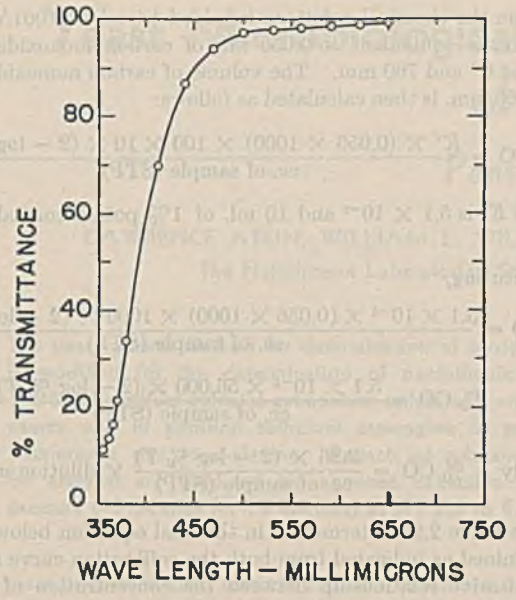

Figure 2. Spectral Transmission Curve of Iodine ($5 \times 10^{-5}N$)

In 1 per cent potassium iodide solution as determined with Coleman photoelectric spectrophotometer (Model 10S) using a 5-mm. slit. Cuvettes were circular tubes of 16.5-mm. diameter and comparison tube was a blank solution (10 cc.) of 1 per cent potassium iodide

centration of absorbing material (iodine in 1 per cent potassium iodide) is linear and the calibration curve is a straight line. This may be expressed as:

$$\text{Concentration} = K' \times \frac{\log T_0}{T}$$

where T_0 equals the intensity of incident light, T equals the intensity of transmitted light (after passing through sample), and K' equals the calibration constant expressing the relationship between concentration of iodine in 1 per cent potassium iodide solution and the logarithm of the percentage transmittance. Absorption of light by iodine ($5 \times 10^{-5}N$) in 1 per cent potassium iodide increases as the wave length of light used decreases into the ultraviolet. As a result of this observation all measurements were made at 350 millimicrons, the point of greatest absorption of the wave-length range available with this type spectrophotometer.

Gas samples are collected in calibrated metallic containers of approximately 1600- to 1700-cc. (STP) capacity; smaller 200- to 250-cc. (STP) glass tonometers may be used for sampling purposes, but give less satisfactory results. The samples are then passed through the iodine pentoxide apparatus by displacement with water, this method being preferable from the standpoint of safety, ease of adjustment, and convenience to the suction method usually employed. It was experimentally observed that the displacement of the 1600- to 1700-cc. sample in 60 minutes followed by a 30-minute flushing with nitrogen gas at the same rate of flow gave most accurate results. However, size of sample, its concentration, and degree of accuracy demanded are all factors which may modify the time necessary to complete an analysis.

For the collection of the liberated iodine the original Gomberg tube used routinely with the iodine pentoxide apparatus was replaced by an absorption tube designed and adapted by J. W. Heim of this laboratory for the present method of analysis (Figure 1). The iodine passes through the collecting tube, which extends almost to the bottom of the absorption vessel, and bubbles out through the sintered-glass filter at its end. The amount of iodide solution used (10 cc.) is sufficient, of course, to keep the filter well below the liquid level. This tube has marked advantages over the Gomberg type, in that its wide-mouthed ground-glass stopper permits ready access to the iodide solution for sampling at the completion of an analysis; it is easily washed out or cleansed; and quantitative results obtained with its use have proved it an efficient type of absorption vessel.

Blank analyses are carried out by filling calibrated containers with nitrogen (commercial), passing the gas through the apparatus, and flushing exactly as with unknown samples. From the amount of iodine measured the equivalent carbon monoxide is then calculated. Blank analyses are always performed prior to the analysis of unknown samples and the blank value in terms of percentage carbon monoxide is always subtracted from the unknown value to obtain the true or "corrected" carbon monoxide percentage concentration. The only reagent required for an analysis is the 1 per cent potassium iodide solution. This should be prepared from a pure grade of potassium iodide (since impure preparations readily liberate appreciable iodine) and should be renewed about once a week. The solution should be stored in a dark glass bottle.

RESULTS

In Figure 2 the spectral transmittance curve of iodine ($5 \times 10^{-5}N$) in 1 per cent iodide solution is presented. Maximum absorption of light by iodine ($5 \times 10^{-5}N$) in 1 per cent potassium iodide solution occurs in the region of the ultraviolet. However, at 350 millimicrons (the lower limit of the Coleman instrument) the absorption is sufficiently great (90 per cent) to provide a means of measurement that is as sensitive and accurate as the routine sodium thiosulfate titration method. Both the titration method and that described here have been employed in this laboratory for determining carbon monoxide in air samples. However, the authors have found the titration method less satisfactory for concentrations of carbon monoxide below 0.005 per cent. Below this value the titration method was less convenient and more time-consuming than the spectrophotometric.

The relationship between the concentration of iodine over a wide concentration range and the logarithm of percentage transmittance is linear. The value of the calibration constant, K', which expresses this relationship between concentration in equivalents per liter and logarithm of percentage transmittance, is 5.1×10^{-5}.

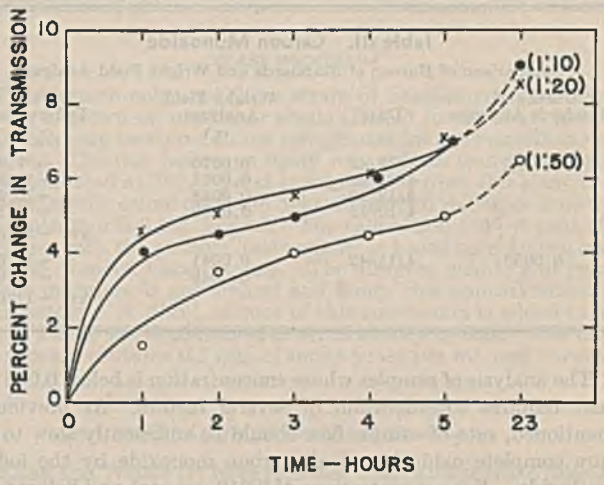

Figure 3. Changes in Percentage Transmission of Iodine in 1 Per Cent Potassium Iodide as a Function of Time

The change in the percentage light transmission of iodine in 1 per cent potassium iodide solution as a function of time is illustrated in Figure 3 and the quantitative effects of such changes are presented in Table I. Dilutions of 1 to 50 showed changes equivalent to only a 6 per cent decrease in the percentage of carbon monoxide after one hour. If spectrophotometric readings are made within 5 to 10 minutes following the completion of the sample run, the error due to any change in light transmission may be considered negligible.

Blank analyses using nitrogen gas (commercial) and performed as previously described gave fairly consistent values, the average being equivalent to a carbon monoxide concentration of 0.0004 per cent. These results are summarized in Table II.

Table I. Effect of Changes in Percentage Transmission of Iodine in 1 Per Cent Potassium Iodide Solution with Time

Dilution	Original Concentration CO	1 Hour CO	1 Hour Change	5 Hours CO	5 Hours Change	23 Hours CO	23 Hours Change
	%	%	%	%	%	%	%
1:10	0.0137	0.0120	−12.4	0.0109	−20.4	0.0103	−24.8
1:20	0.0136	0.0120	−11.8	0.0112	−17.6	0.0107	−21.4
1:50	0.0116	0.0109	− 6.0	0.0091	−21.6	0.0085	−26.8

Table II. Blank Analyses

[Nitrogen (Commercial) Gas Samples]

Date	Sample Volume Cc.	CO (as Iodine Equivalent) %
12/5/42	1660	0.0003
12/5/42	1610	0.0007
12/8/42	1630	0.0002
12/12/42	1590	0.0002
12/12/42	1600	0.0005
12/12/42	1580	0.0004
12/19/42	1640	0.0003
1/2/43	1630	0.0005
1/6/43	1650	0.0004
		Av. 0.0004MD = (±)0.0001

An opportunity to check the accuracy and sensitivity of this method was offered when known standard samples of carbon monoxide mixtures prepared and analyzed by the National Bureau of Standards, Washington, D. C., were sent to Wright Field for check analyses. The comparative results are presented in Table III. In general, values with the spectrophotometric method varied from 4 to 18 per cent below those reported by the Bureau of Standards, with results averaging 10 to 11 per cent lower. While the method is capable of detecting concentrations as low as 0.001 per cent, the limit of accuracy is approximately 5 to 10 per cent for concentrations below 0.010 per cent.

Table III. Carbon Monoxide

(Comparison of Bureau of Standards and Wright Field Analyses)

Bureau of Standards Analyses %	Date	Wright Field Analyses %	Difference %
0.0076	4/12/42	0.0070	− 7.9
	4/12/42	0.0065	−14.5
	4/12/42	0.0068	−10.5
	4/12/42	0.0069	− 9.1
			Av. −10.5
0.0050	4/11/42	0.0041	−18.0
		0.0048	− 4.0
			Av. −11.0

The analysis of samples whose concentration is below 0.010 per cent requires consideration of several factors. As previously mentioned, rate of sample flow should be sufficiently slow to ensure complete oxidation of all carbon monoxide by the iodine pentoxide. For concentrations of 0.010 per cent and below total sampling periods of 80 to 90 minutes gave satisfactory results. Sample volume is another factor to be given consideration when accurate results are desired. Comparative results of analyses of a standard carbon monoxide mixture when using small and large sampling volumes are shown in Table IV. Accuracy and dependability of analysis are obviously increased when larger volumes are used, particularly when the concentration of carbon monoxide is below 0.010 per cent.

The photoelectric colorimeter used with a filter which transmits the greater portion of its light as nearly as possible within the region of the maximum absorption of iodine in 1 per cent potassium iodide solution—i.e., about 350 millimicrons—can be used if the spectrophotometer is not available. A calibration curve must be determined and the sensitivity of the photoelectric colorimetric procedure is somewhat less than that obtained with the spectrophotometer.

From the chemical reactions involved 1.0 ml. of 0.001N iodine solution is equivalent to 0.056 ml. of carbon monoxide measured at 0° and 760 mm. The volume of carbon monoxide at 0° and 760 mm. is then calculated as follows:

$$\% \text{ CO} = \frac{K' \times (0.056 \times 1000) \times 100 \times 10 \times (2 - \log \% T)}{\text{cc. of sample (STP)}}$$

where K' is 5.1×10^{-5} and 10 ml. of 1% potassium iodide are used.

substituting,

$$\% \text{ CO} = \frac{5.1 \times 10^{-5} \times (0.056 \times 1000) \times 1000 \times (2 - \log \% T)}{\text{cc. of sample (STP)}}$$

or

$$\% \text{ CO} = \frac{5.1 \times 10^{-5} \times 56{,}000 \times (2 - \log \% T)}{\text{cc. of sample (STP)}}$$

Finally

$$\% \text{ CO} = \frac{2.86 \times (2 - \log \% T)}{\text{cc. of sample (STP)}} \times \text{dilution used}$$

The value 2.86 is termed K in the final equation below and is determined as indicated from both the calibration curve and the quantitative relationship between the concentration of carbon monoxide and iodine released in the oxidation reaction. The final general equation is then:

$$\% \text{ CO} = \frac{K \times (2 - \log \% T)}{\text{cc. of sample (STP)}} \times \text{dilution}$$

As an example of the use of the above equation in calculating results, the following analytical data are presented:

A 10-cc. portion of the 1 per cent potassium iodide was used to collect the iodine liberated when a gas sample of 2360 cc. (STP) was passed through the apparatus. A 1 to 100 dilution of the iodine collected in the 10-cc. portion of the 1 per cent potassium iodide solution was made and when measured (at 350 millimicrons) with the spectrophotometer against a 10-cc. 1 per cent potassium iodide blank set at 100 per cent light transmission, gave a percentage light transmission of 44.5. The calculation, by substitution of the above data, is illustrated as follows:

$$\% \text{ CO} = \frac{2.86 \times (0.352)}{2360} \times 100 = 0.043$$

Table IV. Standard Carbon Monoxide Mixture

[Comparison of Analyses Using Small and Large Sample Volumes of Standard CO (0.012 per cent)]

Date	Gas Volume Cc.	CO Found %	Deviation %
10/23/42	264	0.011	+0.001
10/23/42	264	0.009	−0.001
10/23/42	264	0.013	+0.003
10/24/42	270	0.006	−0.004
10/24/42	270	0.010	0.000
10/24/42	270	0.006	−0.004
10/24/42	270	0.015	+0.005
		Av. 0.010	MD = (±) 0.0026
10/26/42	1660	0.013	+0.002
10/26/42	1660	0.010	−0.001
10/26/42	1660	0.011	0.000
10/27/42	1660	0.012	+0.001
10/27/42	1660	0.011	0.000
10/27/42	1660	0.013	+0.002
10/30/42	1660	0.011	0.000
10/31/42	1660	0.011	0.000
11/13/42	1645	0.012	+0.001
		Av. 0.011	MD = (±) 0.0008

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Yeast Microbiological Methods for Determination of Vitamins

Pantothenic Acid

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The yeast growth method for determination of pyridoxine is modified for the determination of pantothenic acid. The basal medium contains ammonium sulfate as a nitrogen source and in addition sufficient asparagine to prevent interference due to β-alanine. Extracts of substances to be assayed are prepared by aqueous extraction under pressure (15 pounds for 15 minutes) at pH 5.6 to 5.7, by enzyme digestion at the same pH, or by enzyme digestion followed by aqueous extraction (15 pounds for 15 minutes). The choice of extraction method depends upon the substance, since some have pantothenate in a bound form whereas others do not. The results of assays of a number of representative substances compare favorably with results obtained by other methods.

THE yeast microbiological method recently described for the determination of pyridoxine (*2*) can, with certain modifications, be used to determine pantothenic acid. The yeast growth factor activity of pantothenic acid was known before its need in animal nutrition was established (*12*). That the yeast method was not further developed as an assay method by the discoverers of pantothenic acid was due in part to the interference of β-alanine (*8*). β-Alanine is a structural part of the vitamin molecule but is itself without vitamin activity for higher animals, although under certain conditions it can replace pantothenic acid as a yeast growth factor. The inclusion of asparagine in the yeast growth medium tends to reduce the effect of β-alanine (*13*), but apparently it was not realized that the presence of sufficient asparagine further reduces the interference to an insignificant level. Asparagine does not, however, affect the activity of pantothenic acid under the conditions employed.

The assay method described here has been used by the authors for some time and may possess certain advantages over the microbiological method of Pennington, Snell, and Williams (*8*). The method is rapid, 16 to 18 hours being allowed for yeast growth, and it is especially adapted for turbidimetric measurement of the yeast growth with a photoelectric colorimeter. Furthermore, it offers an opportunity for checking assay results with a different type of microorganism.

APPARATUS

The apparatus employed has previously been described (*1*, *2*). The utility of the Evelyn photoelectric colorimeter was also studied. Using the test tubes usually supplied with the Evelyn and the 660 (red) filter, the absorption curves were found to be essentially the same as with the Lumetron instrument.

SOLUTIONS

SUGAR AND SALTS SOLUTION. One liter contains 200 grams of C.P. dextrose (anhydrous), 2.2 grams of monopotassium phosphate, 1.7 grams of potassium chloride, 0.5 gram of magnesium sulfate ($MgSO_4.7H_2O$), 0.5 gram of calcium chloride ($CaCl_2.2H_2O$), 0.01 gram of manganese sulfate, and 0.01 gram of ferric chloride.

POTASSIUM CITRATE BUFFER. One liter contains 100 grams of potassium citrate ($K_3C_6H_5O_7.H_2O$) and 20 grams of citric acid ($H_3C_6H_5O_7.H_2O$).

THIAMINE SOLUTION, 10 micrograms per ml.

PYRIDOXINE SOLUTION, 10 micrograms per ml.

INOSITOL SOLUTION, 1 mg. per ml.

BIOTIN SOLUTION. S. M. A. Corp. biotin concentrate No. 5000, diluted so that it contains approximately 0.8 microgram of biotin per ml. On one occasion this material was found to contain appreciable amounts of pantothenate and as a consequence a high blank, 15 to 20 per cent absorption, was obtained. The pantothenate was readily destroyed by alkaline heat treatment and the blank or zero value returned to the previous value (less than 10 per cent). Pure biotin was also tried when it recently became available and the results indicate that it may be substituted for the crude concentrate.

AMMONIUM SULFATE SOLUTION, 150 mg. per ml.

ASPARAGINE SOLUTION. One hundred milliliters contain 3 grams of *l*-asparagine. Heating to 100° C. is necessary to dissolve the asparagine.

The solutions are sterilized by heating in flowing steam for 30 minutes on three consecutive days, and may then be stored at room temperature until used. The pyridoxine solution is carefully protected from light.

PANTOTHENATE STANDARD SOLUTION. *d*-Calcium pantothenate (synthetic) is used as a primary standard. A freshly dissolved solution containing 1 mg. per ml. is kept in the refrigerator and used as a working standard for not more than 2 to 3 weeks. Immediately before use a portion of the working standard is diluted with distilled water, to contain 0.1 microgram (100 millimicrograms) per ml.

YEAST INOCULUM

Fleischmann culture 4228, a strain of *Saccharomyces carlsbergensis*, is grown on malt agar slants (Difco) for 24 hours at 30° and then may be stored in the refrigerator for not more than one month. The day before an assay run, a fresh transfer is made and incubated at 30°. Yeast is transferred from this slant to a tube of sterile saline until the light absorption indicates that the concentration is 3 mg. per ml. The calibration (50 per cent absorption with the authors' instrument) is based on a known suspension of moist baker's yeast. The absolute quantity of yeast in the inoculum is not critical and hence this approximation is satisfactory. A 10-ml. aliquot of this suspension is added to 90 ml. of sterile saline contained in an Erlenmeyer flask. The final suspension contains 0.3 mg. of moist yeast per ml. and is ready for use.

PREPARATION OF SAMPLES FOR ASSAY

Kuhn and Wendt (*7*) have reported that pantothenate (filtrate factor) may occur as part of a nondialyzable complex of high molecular weight. The pantothenate content of tissues and yeast, as measured by microbiological methods, is increased by autolysis or enzyme digestion and the increase appears to be due to a decomposition of the complex. Pantothenate is a relatively unstable compound, being readily hydrolyzed by either acid or base. Consequently it is desirable to prepare extracts for assay by the mildest and most direct means. In general, there are three extraction methods available:

1. Enzyme digestion by clarase or other suitable enzyme preparations. Autolysis (self-digestion) may be considered a form of enzyme digestion but is inapplicable to most substances and uncertain with some tissues.
2. Water extraction at high temperature (autoclave) and at the most stable pH range.
3. Enzyme digestion followed by water extraction at high temperature.

Table I. Typical Protocol

To each tube are added 5 ml. of basal pantothenic acid–free medium plus ingredients noted below. After the 10-minute heat treatment 1 ml. of yeast suspension (0.3 mg. of moist yeast) is added to each. The tubes are then shaken at 30° for 16 hours, the turbidity is measured, and the tubes are returned to the incubator for 2 hours and measured again.

No.	H_2O	Added	16 Hours Absorption	16 Hours Pantothenate	16 Hours Pantothenate	18 Hours Absorption	18 Hours Pantothenate	18 Hours Pantothenate	Average
	Ml.	*Ml.*	%	*Mγ/tube*	*γ/g. or γ/ml.*	%	*Mγ/tube*	*γ/g. or γ/ml.*	*γ/ml. or γ/g.*
1	4.0	0	6	...	...	7	...	...	...
2	3.5	0.5 = 50 *Mγ* of calcium pantothenate[a]	16	...	...	21	...	...	...
3	3.0	1 = 100 Same	29	...	...	37	...	...	...
4	2.5	1.5 = 150 Same	41.5	...	...	48	...	...	...
5	2.0	2 = 200 Same	48.5	...	...	55	...	...	...
6	1.0	3 = 300 Same	59.5	...	...	66	...	...	...
7	0	4 = 400 Same	66.5	...	...	73	...	...	...
8	3	1 = 0.025 ml. whole urine[b]	21	70	2.8	28.5	72	2.9	2.7
9	2	2	31	130	2.6	36.5	130	2.6	...
10	1	3	48	195	2.6	55.5	203	2.8	...
11	0	4	54.5	250	2.5	61.5	250	2.5	...
12	3	1 = 0.1 mg. yeast extract (dry)[c]	17.5	57	570	22.5	55	550	550
13	2	2	31.5	110	550	40	112	560	...
14	1	3	43.0	165	550	53	165	550	...
15	0	4	50.5	215	538	57	212	530	...
16	3	1 = 0.5 mg. dry 200-B yeast[d]	22.5	75	150	29	75	150	163
17	2	2	41.5	155	155	48.5	155	155	...
18	1	3	56	265	177	61.5	250	166	...
19	0	4	63	355	173	67.5	317	159	...
20	3	1 = 10 mg. wheat-hard winter No. 2[e]	25.5	87	8.7	32.5	86	8.6	8.3
21	2	2	41.5	157	7.9	49.5	160	8.0	...
22	1	3	54.5	250	8.3	60	235	7.8	...
23	0	4	62.5	350	8.8	67.5	320	8.0	...

[a] Calcium pantothenate, prepared from 1 mg. per ml. of refrigerated solution.
[b] No treatment, preserved at pH 5.6 for 24 hours, 10 ml. diluted to 400 ml.
[c] Digested with clarase for 2 days at 45° C., 100 mg. diluted to 1 liter.
[d] Digested with clarase for 2 days at 45° C., 100 mg. diluted to 200 ml., solution centrifuged until clear.
[e] Water extraction, autoclaved 15 minutes at 16 pounds pressure, 1 gram diluted to 100 ml.

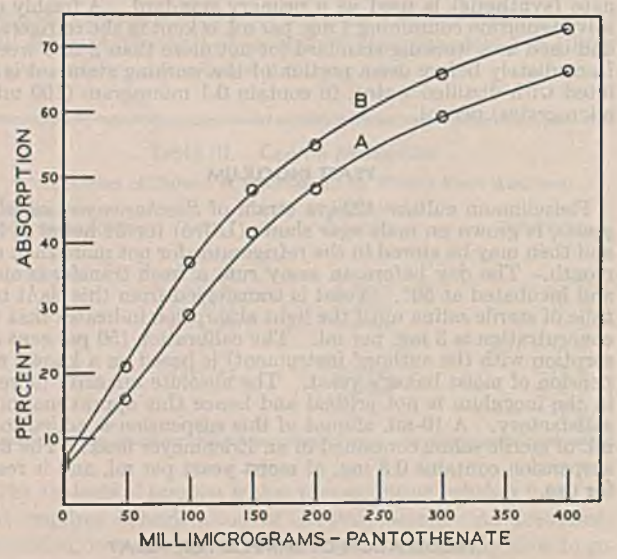

Figure 1. Reference Curve
A. 16 hours *B.* 18 hours

The third method has been found necessary with certain materials—e.g., fresh green peas—because of the presence of a substance inhibitory to yeast growth which, however, is inactivated by a short treatment in the autoclave.

Enzyme Digestion. Insoluble substances should be powdered or dispersed in water with a Waring Blendor or its equivalent. Weigh or measure a portion of the unknown estimated to contain 10 to 20 micrograms of pantothenate into a 40-ml. test tube graduated at 10 and 20 ml. Add 1.0 ml. of the buffer and sufficient water to make the volume to 10 ml. Heat in flowing steam for 5 minutes, cool, and add a weighed amount of clarase roughly equal to the dry weight of the sample. Dissolve by gentle shaking and after adjusting the volume to 20 ml. add 0.5 ml. of benzene. Cork securely and incubate at 45° for 2 days or 37.5° for 3 days. Make the volume to 200 ml. with water. With materials of high potency it is not convenient to weigh out an amount containing only 10 to 20 micrograms: hence 100 mg. are weighed out and after digestion a greater dilution is made. The solutions are centrifuged, if necessary, to obtain a clear extract.

Water Extraction (High Temperature). Some cereals do not appear to require enzyme digestion (*9*, *10*)—e.g., wheat and wheat products—and for these water extraction may be used. Suspend an amount of sample estimated to contain 5 to 10 micrograms of pantothenate—e.g., 1 gram of wheat—in 80 ml. of water, add 1 ml. of buffer, and adjust the pH to 5.6 to 5.7, using dilute sodium hydroxide or sulfuric acid. Heat the suspensions in an autoclave at 7 kg. (15 pounds) for 15 minutes, cool, and dilute to 100 ml. This treatment does not always yield a clear extract even after centrifuging. A short incubation at 45° for 15 minutes after the addition of a knife point of clarase will usually produce flocculation and a clear supernatant fluid.

Enzyme Digestion Followed by Water Extraction. Proceed exactly as in the simple enzyme digestion but rinse the contents of the test tube into a flask with 60 ml. of water, check the pH and adjust, if necessary, to 5.6 to 5.7, and then heat at 15 pounds for 15 minutes. Cool and dilute as usual.

METHOD

Five milliliters of basal pantothenate-free medium plus a solution of the unknown or an aliquot of the pantothenate standard solution are placed in a series of test tubes together with sufficient water to make the volume in each tube 9 ml. The tubes are plugged with cotton, steamed for 10 minutes, cooled, and inoculated with 1 ml. each of the yeast inoculum. The tubes are then shaken at 30° C. and the yeast growth is estimated at 16 and 18 hours by turbidimetric measurements made directly on the tubes with the photoelectric colorimeter. Each assay run includes a series of tubes which are used to construct the reference curve. This series is made with the following levels: 0, 50, 100, 150, 200, 300, and 400 millimicrograms per tube. For assay runs containing more than 25 tubes two reference series are included, one at the beginning and one at the end of the run, and the results of the two are averaged to construct the reference curve.

The basal medium for 20 assay tubes is prepared by mixing the stock solutions in the following proportions: sugar and salts solution, 50 ml.; potassium citrate buffer, 10 ml.; inositol solution, 5 ml.; ammonium sulfate solution, 5 ml.; thiamine solution, 5 ml.; pyridoxine solution, 5 ml.; biotin solution, 5 ml.; asparagine solution, 12.5 ml.; and water to 100 ml.

It is not essential to prepare this medium fresh for each run. Larger batches may be prepared and stored at a temperature a few degrees below 0° for as long as 3 months with no observed ill effects upon the assay run.

The protocol of a typical assay run in which representative materials were assayed is shown in Table I and the reference curve is given in Figure 1. In practice this curve is plotted on ordinary graph paper and values for the unknown are obtained by interpolation. The estimated potency is an average of the value at each assay level and at both 16 and 18 hours. The average deviation from the mean is about 4 per cent. If more than two of the eight values obtained for each assay deviate from the mean by more than 10 per cent, the assay is usually repeated.

Results are reported as pantothenate content and are based on *d*-calcium pantothenate as the primary standard without conversion to the equivalent weight of free acid. There seems to be good precedent for this in the use of thiamine hydrochloride and pyridoxine hydrochloride equivalents without conversion to the equivalent weight of free base.

Table II. Determination of Pantothenate in Presence of Its Hydrolytic Products

Composition of Mixture		Assay	
Completely hydrolyzed calcium pantothenate	Calcium pantothenate	Calcium pantothenate (by assay)	Recovery of added calcium pantothenate
Mg.	*Mg.*	*Mg.*	%
0	1.0	1.02	102
0.25	0.75	0.70	93
0.50	0.50	0.50	100
0.70	0.30	0.29	97
0.80	0.20	0.19	95
0.90	0.10	0.10	100
0.95	0.05	0.055	110
1.00	0.00	<0.01	...

RESULTS

PANTOTHENATE ESTIMATION IN THE PRESENCE OF β-ALANINE. Since β-alanine is a potential interfering substance in this assay it is desirable to determine the limits of the interference. There is no evidence that β-alanine occurs as such in nature except as a degradation product of pantothenate. Consequently, the influence of β-alanine on the assay was studied by analyzing a series of mixtures representing pantothenate in various stages of hydrolysis.

A solution of completely hydrolyzed pantothenate was prepared by heating 10 mg. of the calcium salt dissolved in 10 ml. of 1*N* and 4*N* sodium hydroxide, respectively, at 15 pounds pressure for 2 hours and then cooling and neutralizing. The pantothenate remaining was determined after adding a known amount of pantothenate to the solution. The assay showed 1.5 per cent of the pantothenate left in the 1*N* treated solution and less than 1 per cent in the 4*N* treated solution.

In order to determine the amount of β-alanine produced by the hydrolysis the basal medium was modified by the omission of asparagine, the inclusion of *l*-leucine, and the substitution of pure biotin for the crude which contains β-alanine. A reference series of growth tests with increasing amounts of β-alanine then supplied data for a reference curve, which was used to determine the β-alanine content of the hydrolyzates. Theoretically each 100 parts of calcium pantothenate should yield 37.3 of β-alanine. The 1*N* solution assayed 32.5 parts or 87 per cent and the 4*N* solution 30.25 parts or 81 per cent. Apparently a portion of the β-alanine is destroyed in the course of the hydrolysis.

Assuming that the 4*N* solution represented "completely" hydrolyzed pantothenate, the experiment described in Table II was made. The hydrolyzate was mixed in various proportions with freshly dissolved pantothenate and the mixtures were assayed as unknowns. The results show that little interference from β-alanine is to be expected until 95 per cent or more of the pantothenate has been destroyed.

The ease with which the present method may be converted to a method for β-alanine determination suggests that it may be useful in determining the nature of the loss of pantothenate activity in the processing of vitamin concentrates.

INFLUENCE OF pH ON THE STABILITY OF PANTOTHENATE. Since pantothenate is readily hydrolyzed by either acid or alkali, it is desirable to establish the pH range of maximum stability, so that extracts for assay may be made with a minimum of loss.

Solutions of pantothenate were heated at 7 kg. (15 pounds) pressure for 15 minutes and at 9 kg. (20 pounds) for 1 hour. The heating at 20 pounds was used to accentuate the destruction; the milder heating is used in the assay. The solutions contained in 1 liter: 1 mg. of calcium pantothenate, 10 ml. of potassium citrate buffer, and enough citric acid to adjust the pH to 5.0. Potassium hydroxide (30 per cent) was added to adjust the pH to various levels and aliquots of the solution were removed at each level. After heating and cooling, the solutions were diluted to correspond to a concentration of 100 millimicrograms per ml. and assayed by the usual procedure.

As can be seen from Figure 2, the most stable region is between pH 5.5 and 6.0. In practice the pH is adjusted to between 5.6 and 5.7 in the extraction procedures. Although a suspension of whole wheat had a pH of 6.4, extraction without lowering the pH showed no significant loss. Relatively large quantities of organic matter may have a protective action on pantothenate. It is desirable, however, to adjust the pH to the most stable range in routine analysis, unless investigation shows it to be unnecessary. Clarification of aqueous extracts of starchy substances by the short clarase method proceeds best at pH 5.6 to 5.7.

ENZYMATIC DIGESTION. Early pantothenate assays of certain substances by the microbiological methods did not agree with chick assays (*9*). The discrepancy is now believed to be due to the existence of a bound form of pantothenate available to the chick but not to the microorganism. Digestion with various enzyme preparations liberates the bound pantothenate and tends to bring the assay results by the two methods into somewhat better agreement. Waisman and Elvehjem (*11*) found pancreatin satisfactory, Cheldelin *et al.* (*3*) prefer takadiastase, whereas Strong, Feeney, and Earle (*9*) recommend clarase. The authors have compared clarase with a few other preparations and find it satisfactory and furthermore relatively low in color and in pantothenate content. The product is labeled "Diastase, Vera, highly concentrated 'Clarase' ", and may be obtained from Eimer and Amend, New York. The pantothenate content of this preparation varies between 2 and 4 micrograms per gram. When clarase treatment is used, the results of the assay must be corrected for the pantothenate content of the enzyme.

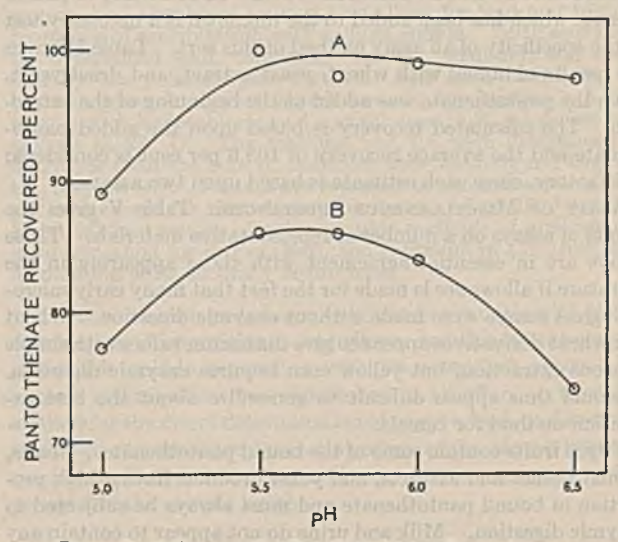

Figure 2. Influence of pH on Stability on Pantothenate
A. 15 pounds for 15 minutes
B. 20 pounds for 1 hour

The enzyme is active in the pH range of maximum stability of pantothenate. Maximum liberation of bound pantothenate was obtained by digestion for 3 days at 37° C. or 2 days at 45° C. Higher temperatures did not appear to offer any advantage. It is essential to maintain an excess of benzene or toluene in the digestion mixture during incubation.

Table III. Alkaline Hydrolysis of Pantothenate

Sample	Original Pantothenate Content	Residual Pantothenate	Destruction
	γ/g.	γ/g.	%
Yeast extract	509	2.5	99.5
	509	5.0	99.0
Dried yeast	164	10.0	94.0
Urine	3.2 γ/ml.	0.06 γ/ml.	98.4
Calcium pantothenate	1 mg.	10.0 γ/mg.	99

Table IV. Recovery of Pantothenate

	Total	Added	Found	Recovered	Recovery
	γ/g.	γ/g.	γ/g.	γ/g.	%
Wheat	8.3	8.0	17.4	9.1	116
	8.3	8.0	16.4	8.1	101
Yeast extract	550	500	1053	503	101
	550	500	1080	530	106
Dried yeast	164	200	371	207	104
	164	200	357	193	97
					Av. 103.6

Hydrolysis of Pantothenate. The relative ease with which pantothenate activity is destroyed by hydrolysis suggests an added test of the specificity of the assay method. Quantitative destruction of the pantothenate activity by alkaline hydrolysis would indicate the absence of an alkali-stable, nonspecific growth factor in the preparation under study. Similarly, acid hydrolysis would indicate the absence of an acid-stable factor.

Yeast extract (100 mg.) and dry yeast (100 mg.) were subjected to clarase digestion and then 10*N* sodium hydroxide was added to make the final concentration 1*N*. Urine and calcium pantothenate were prepared in similar fashion but without digestion. All were heated at 20 pounds for 2 hours, cooled and neutralized, and then assayed for pantothenate after superimposing a known quantity of pantothenate on the test. The results are shown in Table III. It is apparent that essentially all the activity is destroyed by alkaline hydrolysis. Similar results were obtained by acid hydrolysis.

Recovery of Added Pantothenate. The recovery of pantothenate which has been added to the unknown is a necessary test of the specificity of an assay method of this sort. Table IV gives the results obtained with wheat, yeast extract, and dried yeast, when the pantothenate was added at the beginning of the extraction. The calculated recovery is based upon the added pantothenate and the average recovery of 103.6 per cent is considered satisfactory, since each estimate is based upon two assays.

Assay of Miscellaneous Substances. Table V gives the results of assays on a number of representative materials. These assays are in essential agreement with those appearing in the literature if allowance is made for the fact that many early microbiological assays were made without enzymic digestion. Wheat and wheat derivatives appear to give maximum values with simple aqueous extraction, but yellow corn requires enzymic digestion. It would thus appear difficult to generalize about the best extraction method for cereals.

Citrus fruits contain some of the bound pantothenate. Meats, animal tissues and extracts, and yeast products have a high proportion of bound pantothenate and must always be subjected to enzymic digestion. Milk and urine do not appear to contain any significant proportion of bound pantothenate. Enzyme digestion of fresh whole milk does not yield results as high as aqueous extraction. There are indications that fresh milk may have an inhibitory substance which is destroyed by heat. Most fresh vegetables contain bound pantothenate. Fresh green peas contain a substance which is markedly inhibitory to yeast growth but which appears to be completely destroyed when the autoclave method of extraction is employed. Simple enzymic digestion is therefore inadequate and the authors follow the enzyme treatment with autoclaving, when assaying fresh vegetables and fruits.

Table V. Pantothenate Content of Various Substances

Description	Pantothenate Determined: Water extraction (autoclave)	Pantothenate Determined: Clarase digestion	Pantothenate Literature Value
	γ/g.	γ/g.	γ/g.
Cereals			
Whole wheat A	8.9	8.4	12 (4), 12.8 (10)
Whole wheat B	8.3		8.3 (9), 11.2[a] (6)
White flour (patent)	4.0	3.9	3.5 (4), 5.7 (10)
Whole wheat bread (air-dry)	8.9		8.8 (4)
White bread (air-dry)	3.8		6.9 (4)
Yellow corn	4.2	9.3	9.0 (9)
Citrus fruits			
Grapefruit (fresh)	2.7	3.7[b]	2.9 (4)
Oranges (fresh)	1.9	3.3[b]	3.4 (4)
Clarase A	....	1.9	.....
B	....	4.0	.....
C	....	3.3	.....
Meats			
Pork muscle (fresh)	....	8.5	4.7, 5.8 (4)
Pork liver (fresh)	....	66.0	50 (11)
Beef muscle (fresh)	....	8.2	10 (9), 9.8[a] (5)
Beef liver (fresh)	....	59.0	76 (4), 61.5 (9)
Liver concentrate No. 20 (dry)	....	472	.....
	γ/ml. or γ/g.	γ/ml. or γ/g.	γ/ml. or γ/g.
Milk			
Pasteurized A	3.2	....	4.0 (9)
Pasteurized B	3.3	1.8, 2.4[b]	.....
Dried skim milk	43.0	46.0	44, 47 (9)
Milk (whey)[c]	3.2	....	.....
	γ/day	γ/day	γ/day
Urine, normal 24-hour excretion[d]			
Subject A	3410	3250	3000–5000 (9)
Subject A	4400	....	.....
Subject B	4400	....	.....
Vegetables (fresh)			
Tomatoes	2.0	4.5[b]	3.7 (4)
Cabbage	2.1	2.5[b]	1.8 (4)
Beets	1.3	1.6[b]	1.1 (4)
Carrots	2.3	3.1[b]	2.5 (4)
Green peas	3.8	3.9[b]	3.8 (4)
Potatoes	2.1	2.5[b]	3.2 (4)
Yeast			
Brewers' (dry)	....	100	22[e], 86[e] (9)
200-B (dry)	88	164	.....
Yeast extract (dry)	319	500	240[e] (9), 266[e] (8)
Autoclaved (dry)	....	48	.....

[a] Chick assay.
[b] Clarase digestion followed by autoclaving with water.
[c] Casein coagulated with mineral acid and supernatant fluid neutralized and autoclaved for assay.
[d] pH of urine adjusted and assayed without further treatment.
[e] No enzymic digestion.

The specificity of the present method is supported by a number of observations: The values estimated at the various testing levels and at 16 and 18 hours show no significant drift, recovery of added pantothenate is virtually quantitative, the potential interference of β-alanine has been eliminated, the pantothenate activity of extracts prepared for assay may be destroyed by alkaline or acid hydrolysis, and the results obtained are in substantial agreement with reported values obtained by tests which employ other microorganisms, and also in a limited number of cases with the results of the chick assay method.

In order to obtain a measure of the reproducibility of the method, a carefully refrigerated sample of dried yeast was assayed ten times over a period of 3 months. The complete assay including clarase digestion was performed each time. The mean of the ten assays was 166.3 micrograms of pantothenate per gram with an average deviation of 3.2 per cent and a standard deviation of 3.8 per cent.

SUMMARY

A yeast microbiological method for the determination of pantothenate is described. Specificity of response to pantothenate in the presence of β-alanine is obtained by the inclusion of a relatively large proportion of asparagine in the medium in addition to ammonium sulfate. The yeast is grown in test tubes which are shaken at 30° C. for 16 to 18 hours. Yeast growth is estimated with the aid of a photoelectric colorimeter. Methods of extraction of the vitamin have been studied and recovery experiments are described. Extracts of substances to be assayed are prepared by aqueous extraction under pressure (15 pounds for 15 minutes)

at pH 5.6 to 5.7, by enzyme digestion at the same pH, or by enzyme digestion followed by aqueous extraction (15 pounds for 15 minutes).

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Polarographic Determination of Copper, Lead, and Cadmium in High-Purity Zinc Alloys

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A study has been made on the application of the polarographic method of analysis in determining trace elements (down to 1 × 10^{-4} per cent) found in zinc-base die casting alloys. Trace amounts of lead, cadmium, and tin cause intergranular corrosion which results in a serious weakening of the alloy. A polarographic procedure has been developed for the direct determination of copper, cadmium, and lead in these alloys. The samples are dissolved in hydrochloric and nitric acids, evaporated to near dryness, redissolved, treated with hydroxylamine hydrochloride, and finally diluted to volume. The solution is then electrolyzed cathodically over a range of approximately 0.8 volt to obtain waves for copper, lead, and cadmium. Using an 8-gram sample in 50 ml. of solution, these elements can be determined with a precision of ±1 × 10^{-4} per cent of the sample weight. National Bureau of Standards zinc samples have been analyzed using the above procedure and the results found to agree very well with the certificate value. Samples of high-purity zinc and zinc alloys have been analyzed without difficulty. Nineteen elements have been considered from the standpoint of possible interference. The results indicate that trace amounts of copper, cadmium, and lead can be determined polarographically with high precision and accuracy.

METALLURGISTS have found in recent years that traces of lead, cadmium, and tin in zinc-base die-casting alloys tend to cause intergranular corrosion which results in a serious weakening of the alloy. For this reason, specifications for the manufacture of these alloys are very rigid, often requiring that lead shall not exceed 0.003 per cent, cadmium 0.003 per cent, and tin 0.001 per cent. The purpose of this investigation was to develop a system of analysis for zinc die-casting alloys of the Mazak type in which the polarograph could be used with advantage to determine trace amounts down to 10^{-4} per cent with high precision and accuracy.

The determination of trace quantities by wet methods of analysis is exceedingly difficult. It is usually necessary to use large samples (100 grams or more), and to make repeated time-consuming separations. The polarographic method, on the other hand, is particularly suited to trace amounts, the very nature of which greatly reduces the necessity for making separations, and which compares very favorably with the spectrochemical method with respect to the limits of determination possible. A polarographic determination, where applicable, is considerably cheaper than a spectrochemical determination, and can often be carried out more rapidly.

Heyrovský (*4*) in a review of the applications of polarography noted that it is possible to determine lead and cadmium in zinc, and gave curves for a 0.5-gram sample in 5 ml. of hydrochloric acid, in which the concentration of lead is 0.0050 per cent and cadmium is 0.0037 per cent. Terui (*11*) determined lead and cadmium to the nearest 1 × 10^{-3} per cent by dissolving 8 grams of zinc in 70 ml. of 5*N* hydrochloric acid with a few drops of nitric acid and evaporating to 50 ml. Ensslin (*3*) reported a polarographic method for lead and cadmium in pure zinc, in which the zinc was dissolved in nitric acid and the resulting solution combined with different base solutions. The lead and cadmium were determined separately; the lead with an accuracy of 20 per cent of the total amount present, from 3 × 10^{-3} to 5 × 10^{-4} per cent on what would correspond to a 100-gram sample in 1 liter of solution. Krossin (*8*) applied the polarograph to the analysis of copper- and aluminum-bearing zinc alloys for lead and bismuth by means of precipitation with sodium sulfide. Seith and Esche (*9*) determined lead, cadmium, bismuth, thallium, and tin in zinc by the polarographic method. The lead, cadmium, and bismuth were determined simultaneously by treating a 5-gram sample with hydrochloric acid and diluting to 25 ml. before electrolysis at 28° C. The thallium and tin were determined by difference from the sum of cadmium and thallium and lead and tin, respectively. Results are reported to the nearest 1 × 10^{-3} per cent except for tin, which is limited to 1.5 × 10^{-2} per cent. Hohn (*5*) in a review of polarographic methods of analysis outlined a method for copper, lead, and cadmium in zinc, but made no mention of its accuracy or precision.

In the work reviewed above, only one paper deals with the determination of impurities in zinc alloys, and this involves an objectionable sulfide separation with its attendant errors. The present investigation was undertaken in an effort to develop a method for the direct determination of copper, lead, and cadmium in high-purity zinc-base die-casting alloys of the Mazak type with special emphasis on accuracy and precision in the region of 10^{-4} per cent of the sample weight.

APPARATUS AND REAGENTS

The preliminary studies were made with a Leeds & Northrup Electro-Chemograph, and the work was concluded with a Heyrovský polarograph Model XI (E. H. Sargent and Co.). The same capillary was used throughout the investigation. The capillary constant in 2.5*M* zinc chloride was found to be 1.37 $mg.^{2/3}$ $sec.^{-1/2}$ when h = 36.5 cm., t = 3.3 seconds, and temperature = 25 ± 0.5° C. When the curves showed irregularities traceable to fluctuations in the drop time, the capillary was cleaned with concentrated nitric acid as directed by Kolthoff and Lingane (*7*). The pressure on the dropping electrode was maintained by using the Leeds & Northrup electrode assembly in con-

junction with a large flask to serve as a pressure regulator. This kept the pressure constant to within ±0.5 mm. of mercury over periods of not less than 45 minutes. An ordinary electrolysis cell with an internal anode was used throughout these experiments. The step heights were measured by the slope intercept method—i.e., straight lines were drawn along the principal slopes of the curve and the vertical distances between the points of intersection of the extensions of these lines were measured with a millimeter scale (*1*) (see Figure 1). All work was carried out at 25 ± 0.5° C.

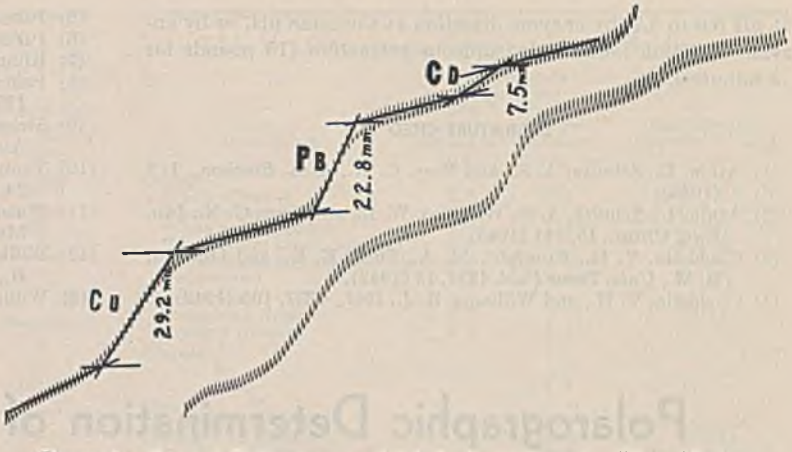

Figure 1. Typical Polarogram and Method of Measuring Wave Heights

Most commercial reagents contain traces of various elements, and it was found necessary to check the purity of the reagents used under the conditions existing in the procedure outlined below. It was not possible to procure zinc metal of such purity that no steps were obtained under the operating conditions. (The word "step" is intended to describe the increase in current caused by the discharge of an ion, *7*.)

A sample of zinc was made uniform by reducing the sample to shavings and mixing. A portion of this sample was analyzed polarographically, using the procedure outlined, without addition of metal ions. This gave the residual current for the ensuing determinations.

Concentrated Hydrochloric Acid (sp. gr. 1.19). One hundred milliliters of concentrated hydrochloric acid were evaporated almost to dryness in a 125-ml. Pyrex beaker. An 8-gram sample of zinc was then added to this residual liquid and the whole carried through the procedure for analysis. After subtracting the residual current due to the zinc, it was found that for the 32 ml. of hydrochloric acid required, a correction of 5×10^{-5} per cent of cadmium and 5×10^{-5} per cent for copper would have to be applied. No trace of lead was found.

Concentrated Nitric Acid (sp. gr. 1.42). One hundred milliliters of concentrated nitric acid were treated in a manner similar to that used for the hydrochloric acid. No traces of copper or lead were found, but cadmium corresponding to 0.00015 per cent in an 8-gram zinc sample was detected. Inasmuch as the amounts of nitric acid used were seldom in excess of 10 ml., this amount of cadmium was considered negligible for the present purpose.

Hydroxylamine Hydrochloride (2*N*). Ten milliliters of 2*N* hydroxylamine hydrochloride were evaporated almost to dryness and treated as was the hydrochloric acid. No traces of copper, lead, or cadmium were found.

Gelatin Solution (0.2 per cent aqueous). One gram of gelatin was ashed in a porcelain crucible and the residue taken up in a few milliliters of concentrated hydrochloric acid. The contents of the crucible were then added to a zinc sample as for hydrochloric acid. Copper corresponding to 3×10^{-4} per cent and cadmium to 1×10^{-4} per cent in an 8-gram zinc sample were found. Since only 2.5 ml. of the 0.2 per cent solution are used in an analysis, these impurities were considered negligible.

Distilled Water. Five hundred milliliters of water were evaporated to dryness and treated as for hydrochloric acid. No detectable amounts of copper, lead, or cadmium were found.

Using the procedure described below, it was found that the over-all effect of impurities in the reagents amounts to copper 0.00005 per cent, lead 0.00000 per cent, and cadmium 0.00005 per cent. These amounts may be neglected for most purposes.

The standard solutions of copper, lead, and cadmium, required for the calibration of the capillary were prepared by diluting stock solutions.

Standard Stock Solution for Copper. A 0.2*M* solution of cupric nitrate was prepared by dissolving 12.714 grams of electrolytic copper in dilute nitric acid and diluting to 1 liter. This solution was analyzed by slow deposition, and found to be 0.1982 ± 0.0002*M* (average of four determinations).

Standard Stock Solution for Cadmium. A 0.2*M* solution of cadmium nitrate was prepared by dissolving 22.496 grams of c.p. cadmium in dilute nitric acid and diluting 1 liter. This solution was analyzed by electrical deposition and found to be 0.1996 ± 0.0004*M* (average of three determinations).

Standard Stock Solution for Lead. A 0.2*M* solution of lead nitrate was prepared by dissolving 41.458 grams of c.p. lead in dilute nitric acid and diluting to 1 liter. This solution was analyzed by the lead acid method and found to be 0.1981 ± 0.0002*M* (average of four determinations).

PROCEDURE

To 8 grams of turnings in a 125-ml. Pyrex beaker add slowly 25 ml. of concentrated hydrochloric acid. After the first violent reaction has subsided, add cautiously a few milliliters of concentrated nitric acid, and warm to effect solution. When solution is complete, add sufficient nitric acid to make the volume of added nitric acid 5 ml. Evaporate on a hot sand bath until salts begin to crystallize out, and the mixture boils like thick sirup. If desired, the evaporation may be hastened somewhat by careful heating on a wire gauze. Allow the mixture to cool for a short time, or until solid. Wash down with distilled water until about 10 ml. of water have been added.

Add 7 ml. of concentrated hydrochloric acid and heat on a sand bath until any hydrolyzed aluminum is redissolved. This may require 10 to 15 minutes. Transfer to a 50-ml. volumetric flask with the minimum of distilled water. Add 2.5 ml. of 0.2 per cent gelatin solution and 0.1 ml. of 2*N* hydroxylamine hydrochloride. Shake and heat until the solution becomes colorless. If necessary, add a second portion of hydroxylamine hydrochloride. (Occasionally a solution may remain colored in spite of a large excess of hydroxylamine. If the excess corresponds to 100 to 200 times the amount of iron present, this color can usually be ignored.) Dilute with freshly boiled distilled water, cool, and dilute of volume. Bubble with nitrogen in the electrolysis cell for 15 to 20 minutes to remove dissolved oxygen, and electrolyze from −0.04 volt to the discharge potential of the supporting electrolyte (approximately −0.8 volt) using a bridge potential of 1 volt. The sensitivity should be adjusted to give the largest possible steps in the curves.

The ferric iron is readily reduced to the ferrous state by treating the warm hydrochloric acid solution with hydroxylamine (*2*). In this state, the iron will not interfere with the determination of the copper, lead, and cadmium. Strubl (*10*) also made use of this reagent in the analysis of zinc blende which was high in iron.

The time required for a determination of copper, lead, and cadmium in a zinc-base alloy using the above procedure is about 3 hours. However, a large number of samples may be run at the same time, as only 10 minutes are required for a polarogram after the sample is prepared.

If copper is present in the alloy in excess of 0.1 per cent, it is advisable to remove the copper by electrodeposition from nitric acid solution as follows:

To 8 grams of zinc in a tall-form 250-ml. beaker, add 50 ml. of water and then add 23 ml. of nitric acid in small portions. When all the acid has been added, boil to complete solution, dilute to 100 ml. (or sufficient volume to cover the electrodes), and electrolyze at 4 amperes and 3 to 4 volts for 1 hour, with a rotating gauze anode and a gauze cathode. At the end of this time, wash down the cover glass and beaker and continue for another 5

minutes. Carefully remove the electrodes, while washing with a heavy stream of water. Under no circumstances should the circuit be broken before the electrode is completely free of acid. Rinse the electrode several times in 95 per cent alcohol, shake free of excess alcohol, and dry by revolving rapidly over a Bunsen flame after igniting the film of alcohol. Weigh as pure copper. Replace the anode, which may have lead oxide deposited, in the electrolyte and heat the whole to boiling. Then wash the electrode and remove it and boil the solution down to incipient crystallization. Add hydrochloric acid and carry out the procedure as for zinc which is low in copper. Care must be taken to remove all excess nitric acid. To do this, an extra evaporation with 10 ml. of hydrochloric acid is recommended before addition of the 7 ml. of hydrochloric acid and 10 ml. of water to redissolve the hydrolyzed aluminum.

This modification increases the total time required for an analysis, but when a large number of samples are to be run, this increase is considerably lessened. The results obtained for the copper by this method are usually slightly high (0.03 per cent high for 3 per cent copper). No traces of cadmium or zinc were found in the deposit when the deposit had been redissolved and deposited, and the electrolyte examined polarographically.

Table I. Calibration Constants for Copper, Lead, and Cadmium

Element	Leeds & Northrup 1	Leeds & Northrup 2	Leeds & Northrup Average	Sargent 1	Sargent 2	Sargent Average
	%/microampere	*%/microampere*	*%/microampere*	*%/microampere*	*%/microampere*	*%/microampere*
Cu	0.01815	0.01864	0.0184	0.02040	0.02078	0.0206
Pb	0.02905	0.03009	0.0296	0.03334	0.03413	0.0338
Cd	0.01869	0.01873	0.0187	0.01875	0.01888	0.0188

RESULTS AND DISCUSSION

The capillary was calibrated by making additions of copper, lead, and cadmium from the diluted stock solutions. The calibration was carried out over a concentration range of $10^{-6}M$ to $3 \times 10^{-4}M$ on both the Leeds & Northrup and the Sargent polarographs. The step heights were obtained by difference from the residual current of the zinc used as a supporting electrolyte, and those produced by the zinc plus added ions. In the lower concentration range (1×10^{-6} to $5 \times 10^{-6}M$), this residual current amounted to from ten to twenty times the increase in current due to the added ions, and, accordingly a large error was introduced. This error was more significant when using the Leeds & Northrup instrument than when employing the Sargent apparatus. The fact that the latter has over twice the maximum sensitivity of the former would account in part for this difference in the results.

The calibration constants for two successive calibrations using two different zinc samples as a supporting electrolyte are given in Table I.

The factors were obtained in terms of per cent per microampere for each metal for the sake of convenience in calculating the values from the step heights. The basis for the calculation is an 8-gram sample in 50 ml. of solution.

In order to determine the precision of the method, and to discover the greatest source of error in the polarographic procedure, five series of determinations were carried out, using the Sargent instrument (Table II).

1. Precision of repeated determinations on the same cell.
2. Precision of repeated determinations on different aliquots of the same solution.
3. Precision of repeated determinations on different samples of the same alloy.
4 and 5. Precision of repeated measurements of the same curve by different individuals and by the same individual.

These results indicate that the mean deviation of measurement in all cases is approximately one half of the total mean deviation of the procedure. The deviation is not significant, however, in that it barely affects the fourth place of decimals. The over-all

Table II. Precision of Polarographic Procedure

Series[a]	Copper Average	Copper Deviation[b]	Lead Average	Lead Deviation[b]	Cadmium Average	Cadmium Deviation[b]
	%	%	%	%	%	%
1	0.0018_1	0.00006	0.0057_9	0.00008	0.0005_4	0.00004
2	0.0018_1	0.00003	0.0058_7	0.00003	0.0006_2	0.00005
3	0.0018_5	0.00011	0.0056_0	0.00008	0.0006_2	0.00005
4	0.0010_1	0.00003	0.0017_1	0.00004	0.0009_3	0.00002
5	0.0010_2	0.00003	0.0016_7	0.00005	0.0009_2	0.00003

[a] Each of series 1, 2, and 3, is result of seven determinations. In series 3, each determination was made in duplicate. Series 4 is result of duplicate measurements by nine different individuals. Series 5 is result of ten measurements of same curve.
[b] Average deviation from arithmetical mean.

precision would indicate that it is possible to determine copper, lead, and cadmium to within 1×10^{-4} per cent for the range of concentrations encountered in high-purity alloys of the Mazak type.

At the time this investigation was carried out, it was impossible to procure a National Bureau of Standards zinc die-casting alloy of the type desired (Mazak 3). In lieu of this, an analysis was made on an alloy high in copper. Analyses are also presented for several standard zinc spelters (Table III).

It has been found, as a result of the analysis of a large number of commercially analyzed zinc samples, that the polarographic results are usually high. No explanation is available for this phenomenon. The calibrations have been checked and rechecked repeatedly in the authors' laboratory by different methods. There is a possibility that because of the small amount of handling, the polarographic results represent a closer approximation to the true value than analyses which are the result of many successive manipulations. The degree of precision of the polarographic procedure is high, as illustrated, and the deviations from other analyses do not show signs of a constant error. This is evidenced by examination of the above results for the Bureau of Standards samples.

Table III. Accuracy of Method

(Sargent instrument)

	Copper	Lead	Cadmium
	%	%	%
	National Bureau of Standards. Sample 94		
Experimental	2.83[a]	0.0318	0.0025
Precision	±0.01	±0.0002	±0.0001
Certificate value	2.82	0.031	0.004
	National Bureau of Standards. Sample 109		
Experimental	0.0007	0.0025	0.0019
Precision	±0.0001	±0.0001	±0.0001
Certificate value	0.0005	0.0020	0.0018
	National Bureau of Standards. Sample 108		
Experimental	0.0004	0.0505	0.0960
Precision	±0.0001	±0.0002	±0.0007
Certificate value	0.0004	0.047	0.092

[a] By electrodeposition.

INTERFERING ELEMENTS

There are some nineteen elements which may be found in zinc, either as impurities, or as alloying elements: Cu, Pb, Cd, Ni, Co, Mn, Ag, As, Hg, Tl, Bi, Sb, Al, Fe, Ge, Ga, In, Mg, and Sn. ("Interference" is intended to describe the preliminary or almost coincidental discharge of some undesired ion which either results in a masking of the step desired or makes impossible the recording of the desired ion at maximum sensitivity.)

Experiments with 0.05 per cent each of nickel, cobalt, manganese, silver, arsenic, mercury, and indium show that there is no detectable effect on the steps for copper, lead, and cadmium within ±0.0001 per cent. Magnesium was tried up to 0.5 per cent and no interference was detected. This is to be expected, since the discharge potential of magnesium is well above that of

zinc. Copper up to 0.1 per cent has been determined polarographically without reducing the sample size while retaining a high degree of precision for lead and cadmium. Results of the electrodeposition of copper in conjunction with the polarographic determination of lead and cadmium indicate that there is no loss of lead and cadmium during this operation and that less than 0.01 per cent of the copper remains after deposition. Bismuth was found to give a step which precedes that of copper and for this reason interferes with the ensuing determination of copper, lead, and cadmium when present in excessive amounts. It is not close enough, however, to mask the copper step. The factor for bismuth was found to be approximately 0.024 per cent per Å. for an 8-gram zinc sample in 50 ml. Antimony at 0.05 per cent gives a poorly defined step which interferes with the steps for copper, lead, and cadmium; however, at 0.01 per cent and less, no interference was found. Thus antimony concentrations of 0.01 per cent can be tolerated. The nature of the interference would seem to indicate a small diffusion coefficient for Sb^{+++++} in this particular medium. Thallium gives a well-defined wave which comes between lead and cadmium in 2.5 zinc chloride. For trace amounts of copper, lead, and cadmium, only 0.002 per cent of thallium can be present. This corresponds to the findings of Seith and Esche (*9*) with regard to the limit of detection of thallium in zinc. Germanium was not tested for interference, because of the extreme volatility of its chloride. Gallium was not tested because of the difficulty in obtaining a salt of this metal. No interference is to be expected from gallium because of its high discharge potential.

Stannic tin when present in amounts greater than 0.0015 per cent will give a measurable increase in the step height for lead. Occasionally, amounts greater than this may be tolerated due to volatilization of stannic chloride, but such amounts of tin are not volatilized appreciably by this particular procedure. Kalovsek (*6*) indicates that the electroreduction of stannic tin is not reversible except in hydrochloric acid solutions of high concentration (above 0.1 *N*), where, however, the reduction process appears inhibited. This would explain why such a large amount of stannic tin would cause no interference. These results confirm those of Seith and Esche for the limit of detection of tin in zinc. Aluminum up to 6 per cent has been found to be without detectable effect on the height of the steps for copper, lead, and cadmium. Higher concentrations of aluminum might have an effect only in so far as they affected the concentration of the supporting electrolyte. Iron, after reduction with hydroxylamine hydrochloride is without significant effect in the determination of copper, lead, and cadmium at 2.5 per cent. It is necessary, of course, to adjust the amount of hydroxylamine hydrochloride used in accordance with the iron content for satisfactory results.

A study of the polarographic determination of trace quantities of tin, aluminum, and magnesium in high-purity zinc alloys is in progress.

CONCLUSION

Trace amounts of copper, lead, and cadmium can be rapidly determined by the polarographic method in high-purity zinc and zinc die-casting alloys with a high degree of precision. Such a method should prove of value in industrial laboratories where time is at a premium.

In an effort to make possible a complete polarographic analysis of high-purity zinc and zinc die-casting alloys, procedures are being developed for the determination of other elements present.

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Determination of Pectin in Biological Materials

Modification of Pentose-Furfural Method

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Pectin is converted to a pentose which produces the furfural with which this method is concerned. Data are presented to show the normal levels of furfural-yielding substances in various organs and fluids from rabbits. The analytical procedures described make it possible to recover 95 to 100 per cent of pectin which has been added to animal tissues and fluids.

PRESENT wartime conditions, which have increased interest in pectin sols for intravenous use in treating shock, have made it necessary to devise a semimicromethod for the determination of pectin in biological materials.

Pectinum N. F. VII which is suitable for intravenous use is essentially a pure polygalacturonic acid ester. When such a pectin is refluxed at elevated temperatures with 12.5 per cent hydrochloric acid the polygalacturonic anhydride units are decarboxylated, producing a mole of carbon dioxide for each carboxyl, and forming furfural from the newly formed pentose. Accurate quantitative procedures based upon the determination of the carbon dioxide evolved and upon the furfural produced have been developed during the forty years which have elapsed since the general reactions were first described by Tollens (*5*).

The methods which have been developed for quantitative estimations of furfural are sensitive to extremely small amounts and adaptable to colorimetric procedures. The carbon dioxide methods are useful only when relatively large amounts of material are available; hence for purposes of determining pectin in biological systems the furfural scheme is preferred.

Furfural methods and their applications to pectin analyses were discussed by Browne and Zerban in 1941 (*2*). Youngburg (*7*) in 1927 described a particular adaptation of furfural estimation useful for biological materials. Bryant, Palmer, and Joseph (*4*) used a modification of Youngburg's method in these laboratories early in 1941 for an examination of the liver and other organs of the rabbit. The Youngburg scheme involved steam distillation from 85 per cent phosphoric acid and colorimetric determination of furfural in the distillate by the furfural–aniline acetate reaction. Details for the use of the Youngburg method,

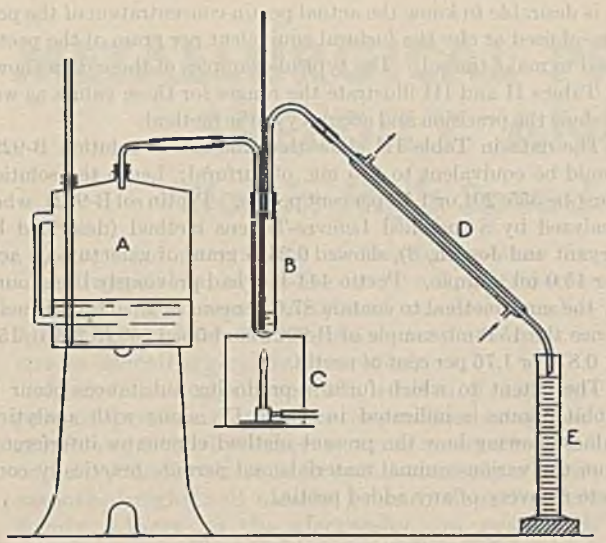

Figure 1. Distillation Apparatus

particularly the step involving trichloroacetic acid treatment of samples, were given by Andersch and Gibson (*1*).

Modifications of the Youngburg method previously published failed to allow reasonable recovery of pectin which had been added to urine, and also failed to provide samples from blood and tissue materials which could be distilled without excessive foaming. The method described here provides for an alcohol treatment of urine samples which permits complete recovery of pectin added to urine. It substitutes sodium tungstate for trichloroacetic acid and centrifuging for filtration, in preparing other samples, and continues with the regular Youngburg distillation. Foaming is eliminated and recoveries from control samples are excellent. The technique for the determination of furfural in the distillate by a photoelectric colorimeter is described. The procedures given below are the result of several hundred analyses of rabbit blood, urine, and organs, and of many mixtures of pectin with these animal materials.

APPARATUS

The distillation apparatus shown in Figure 1 is composed of a steam generator, *A*, made from a large ether can with burner underneath regulated by screw clamp for control of steam flow into *B*, the distillation unit, a Pyrex 25 × 150 mm. test tube, with thermometer covering the range 0° to 200° C.; *C*, tin cylinder shield for microburner; and *D*, a water-cooled condenser, 250-mm. jacket, with inside tube preferably 6 to 7 mm. in diameter and turned down at end for delivery into *E*, a 50-ml. graduated cylinder. An ordinary steam generator made from glass may be used instead of the metal one described.

A number of the Pyrex 25 × 150 mm. test tubes should be available because urine samples are prepared in them and then may be stored until time is available for distillation. It is convenient to have 2.0-, 3.0-, 4.0-, and 5.0-ml. volumetric pipets, as well as Mohr pipets, 1.0-ml. size graduated in 0.10 divisions. A centrifuge at least as large as the International No. 1 and several 30-ml. centrifuge tubes are required. A photoelectric colorimeter is recommended. The Fisher Electrophotometer with a blue filter (No. 425B) and the Klett-Summerson photoelectric colorimeter with a green filter (No. 54) have been used successfully with this method.

REAGENTS

Freshly prepared furfural, vacuum-distilled at a pressure of 20 to 30 mm.

Standard furfural stock solution prepared from the freshly distilled furfural; 1.000 gram is diluted to 500 ml. with distilled water. This stock solution may be kept for several weeks in a refrigerator.

Standard dilute solution of furfural for calibration of photoelectric colorimeter or as a comparison solution with the Duboscq-type colorimeter. This solution is made by diluting 5.0 ml. of the stock to 1000 ml. with distilled water. This dilute standard (1.00 ml. equivalent to 0.01 mg. of furfural) should be made fresh the day of use.

Freshly distilled aniline, free from furfural. Test by adding 0.25 ml. to 2.0 ml. of glacial acetic acid. If a pink color develops within one minute, furfural is present.

Glacial acetic acid, 85 per cent orthophosphoric acid, 10 per cent by weight solution of sodium tungstate ($Na_2WO_4.2H_2O$), 0.5*N* sulfuric acid, and isopropyl or ethyl alcohol, at least 95 per cent.

PREPARATION OF SAMPLES

Pectin Sols for Check on Method. The utility of this method in determining the fate of injected pectin depends upon having an accurate figure for the furfural equivalent per milliliter of the pectin sol being used in the animal experiments. Each lot of Exchange Pectinum N. F. VII will have a definite value for the furfural equivalent, usually varying from 190 to 215 mg. of furfural per gram of pectin. These furfural values, obtained by the method described below, have been checked by using the standard gravimetric phloroglucin method (*6*). The values by the two methods agree almost perfectly.

Animal work with pectin usually involves 1.0 to 2.0 per cent sols which have been sterilized by autoclaving and filtered to sparkling brilliance. Standardization analyses on such sols should be run on samples prepared as follows: 5.0 ml. of 1.0 to 2.0 per cent pectin sol, diluted to 200.0 ml. with distilled water; 2.0 ml. of this diluted sol should be used for each analysis.

Urine. Normal urine from rabbits usually contains so little furfural-yielding material that 4.0-ml. samples are required for an analysis. When pectin has been injected into an animal, it is necessary to use only a 2.0-ml. sample of urine to get good results, while pectin is being excreted.

Pipet duplicate samples of urine into 25 × 150 ml. Pyrex test tubes and add 10 volumes of at least 95 per cent alcohol. After standing for an hour or two centrifuge the tubes and pour off the supernatant liquor. Place the tubes containing the residue in a drying oven at 100° C. and dry for about 30 minutes, or until no odor of alcohol can be detected. These tubes containing the dried residue may then be covered and stored until ready for analysis.

Urine and Added Pectin, as Control on Method. Prepare a check solution by diluting 5.0 ml. of a 1.0 to 2.0 per cent pectin solution with urine up to 200.0 ml. in a volumetric flask, and treat 2.0-ml. samples with alcohol as described above.

Blood. Prepare a Folin-Wu blood filtrate by the following method: Weigh 2.0 ml. of oxalated blood (10 mg. of potassium oxalate per 5 ml. of blood) accurately in a 30-ml. centrifuge tube. Add to this by pipet 13.0 ml. of distilled water, 2.0 ml. of 10 per cent sodium tungstate solution, and 3.0 ml. of 0.5*N* sulfuric acid. Mix the contents of the tubes well, allow the samples to stand 15 minutes, then centrifuge at about 1000 r.p.m. for 30 minutes. Remove the clear centrifugate and save for analyses, using 2.0 ml. for each sample, as described below.

Animal Tissues. Analyses may be made upon the organs immediately after they have been removed from the animal and weighed, or the material may be frozen with dry ice and stored for later use. In either case it is necessary to macerate the organ (or a portion of it in cases of large organs) in a mortar. Transfer as much as possible from the mortar (not to exceed 10 grams of the larger organs), into a tared beaker and then add about 10 times as much distilled water as the weight of the organ used. Stir this weighed mixture thoroughly and transfer to a mixer such as the Waring Blendor where it is reduced to a homogeneous slurry. Pipet a volume of this slurry equivalent to about 0.3 to 0.5 gram of the original organ into a 30-ml. centrifuge tube, and weigh. Add distilled water to bring the volume to 15.0 ml., then add 2.0 ml. of 10 per cent sodium tungstate solution and 3.0 ml. of 0.5*N* sulfuric acid, mixing the contents of the tube by careful swirling after the addition of each reagent. Mix the contents of the tubes well and allow the samples to stand 15 minutes. Then centrifuge at about 1000 r.p.m. for 30 minutes. Remove the clear centrifugate and save for analyses, using 2.0 ml. for each distillation.

METHOD

The sample for analysis should be in a 25 × 150 mm. Pyrex test tube, prepared as discussed under "Preparation of Samples".

Add to the sample in the test tube 5.0 ml. of 85 per cent phosphoric acid and connect with the condenser and the steam generator. Light the microburner and raise the temperature rapidly to 170° to 175° C. The temperature should be maintained at this point and never allowed to go higher during the 20- to 30-minute distillation period.

Collect 40 ml. of distillate in the 50-ml. graduated cylinder used as a receiver, and an additional 10 ml. of distillate in a test tube

previously marked at the 10-ml. level. Test this last distillate for the presence of furfural as follows: Mix 2.0 ml. of the distillate, 0.25 ml. of aniline, and 2.0 ml. of glacial acetic acid in a test tube. If no color develops in one minute, stop the distillation and discard the final 10 ml. of distillate. If color does appear in the test sample, distill an additional 10 ml. and test a portion for furfural as above described. When analyzing blood or urine of untreated animals, 30 ml. of distillate are usually sufficient to contain all the furfural.

Table I. Recovery of Pectin Added to Rabbit Urine

Material Analyzed	Furfural Found Youngburg method	Furfural Found Present method
	Mg.	*Mg.*
2.0 ml. of 1.75% pectin sol B-9221	7.08	7.08
48.0 ml. of urine + 2.0 ml. of pectin sol B-9221	11.46	9.22
48.0 ml. of urine + 2.0 ml. of distilled water	7.33	2.08
Difference, due to added pectin	4.13	7.14
Recovery of added pectin, %	58.3	100.9

Table II. Furfural Equivalent of 1.00 Gram of Pectinum N. F. VII

(Sample 444-H-3. Analyses made on water solution containing 750 mg. of pectin per liter)

Operator	Furfural Found *Mg./gram 444-H-3*
E.F.B.	201
G.H.P.	198
G.H.P.	205
G.H.P.	200
Average value used	201

Combine and mix 20.0 ml. of the original 40-ml. distillate and 5.0 ml. of each 10-ml. distillate showing a positive test for furfural. Place 5.0 ml. of this mixture (or 5.0 ml. of the original 40-ml. distillate when the furfural test in the next 10 ml. was negative) in a tube and mix with 0.5 ml. of aniline and 4.5 ml. of glacial acetic acid, carefully measured with a pipet.

Set this mixture aside in the dark at 20° to 25° C. for exactly 15 minutes, at which time the color intensity is determined with a photoelectric colorimeter. This 15-minute period has been determined experimentally as giving the most exact value. The colorimeter should be calibrated by using the standard dilute furfural solution described above, spacing samples over the range from 0.001 to 0.050 mg. of furfural per 10 ml. of solution containing 0.5 ml. of aniline, 4.5 ml. of glacial acetic acid, and the diluted standard furfural solution.

When using the Duboscq type of instrument a standard for colorimetric comparison must be prepared for each sample. This is done by mixing 2.0 ml. of the dilute standard furfural solution made that day, with 3.0 ml. of distilled water, 0.5 ml. of aniline, and 4.5 ml. of glacial acetic acid. The tube containing this mixture and also the one with the unknown are set aside in the dark for exactly 15 minutes, for color development. The unknown and the standard should have about the same color intensity. If they do not, the solutions should be remade, reducing the volume of either the unknown or standard, adjusting the total volumes with distilled water to keep them the same as outlined above.

DISCUSSION

An illustration of the accuracy of the present method compared with that of the original Youngburg scheme, in the case of rabbit urine, is given by Table I.

It was found that urea added to pectin sols decreased the recovery of pectin by the Youngburg procedure. Many analyses, as illustrated by Table I, show that even though the alcohol precipitation scheme does remove some of the naturally occurring aldehyde-producing substances of urine, it also removes the urea and thereby allows complete recovery of the added pectin. The increase in furfural due to added pectin in urine (human as well as rabbit), using the alcohol precipitation method, has always been found equivalent to the furfural obtained from the pectin when analyzed alone.

When this method is used to follow pectin excretion from animals to which pectin sol transfusions have been given, it is necessary to know the furfural equivalent for the pectin sol used and the normal furfural values for the organs or fluids being examined. It is desirable to know the actual pectin concentration of the pectin sol used or else the furfural equivalent per gram of the pectin used to make the sol. The typical examples of these data shown in Tables II and III illustrate the ranges for these values as well as show the precision and accuracy of the method.

The data in Table III show that 100 ml. of solution B-9221 would be equivalent to 355 mg. of furfural; hence the solution must be 355/201 or 1.76 per cent pectin. Pectin sol B-9221, when analyzed by a modified Lefevre-Tollens method (described by Bryant and Joseph, *3*), showed 0.2315 gram of galacturonic acid per 15.0-ml. sample. Pectin 444-H-3 had previously been found by the same method to contain 87.6 per cent of galacturonic acid; hence the 15.0-ml. sample of B-9221 contained 100 (0.2315)/15.0 × 0.876 or 1.76 per cent of pectin.

The extent to which furfural-producing substances occur in rabbit organs is indicated in Table IV, along with analytical values showing how the present method eliminates interference from the various animal materials and permits practically complete recovery of any added pectin.

Table III. Furfural Equivalent of 1.00 Ml. of Autoclaved 1.75 Per Cent Isotonic Pectin Sol

(Sol B-9221 made from Pectin 444-H-3. Analyses made on dilution of 5.0 ml. of B-9221 to 200 ml. total)

Operator	Date	Furfural *Mg./ml. B-9221*
G.H.J.	Sept. 11, 1942	3.59
G.H.P.	Sept. 14, 1942	3.46
E.F.B.	Oct. 5, 1942	3.54
G.H.J.	Oct. 7, 1942	3.57
E.F.B.	May 27, 1943	3.58
G.H.J.	July 21, 1943	3.54
E.F.B.	July 21, 1943	3.56
Average value		3.55

Table IV. Furfural Obtained from Rabbit Tissues and Recovery of Pectin Added to These Tissues

(1.00 ml. of pectin sol B-9221 added is equivalent to 3.55 mg. of furfural, Table III)

Material	Sample Weight	Furfural Obtained Original tissue sample	Furfural Obtained Tissue plus 1.0 ml. of pectin sol B-9221	Furfural Due to Added Pectin	Recovery of Added Pectin
	Grams	*Mg.*	*Mg.*	*Mg.*	%
Blood	1.903	0.23			
	1.539		3.80	3.61	101.7
Bile	0.320	0.29			
	0.320		3.71	3.42	96.3
Bone	0.500	0.10			
	0.500		3.62	3.52	99.2
Heart	0.330	0.14			
	0.330		3.62	3.48	98.0
Kidney	0.535	0.22			
	0.535		3.74	3.52	99.2
Liver[a]	0.492	1.95			
	0.496		5.60	3.65	102.8
Spleen	0.320	0.14			
	0.320		3.62	3.48	98.0

[a] Better results are obtained when sample weight of liver is about 0.2 to 0.3 gram.

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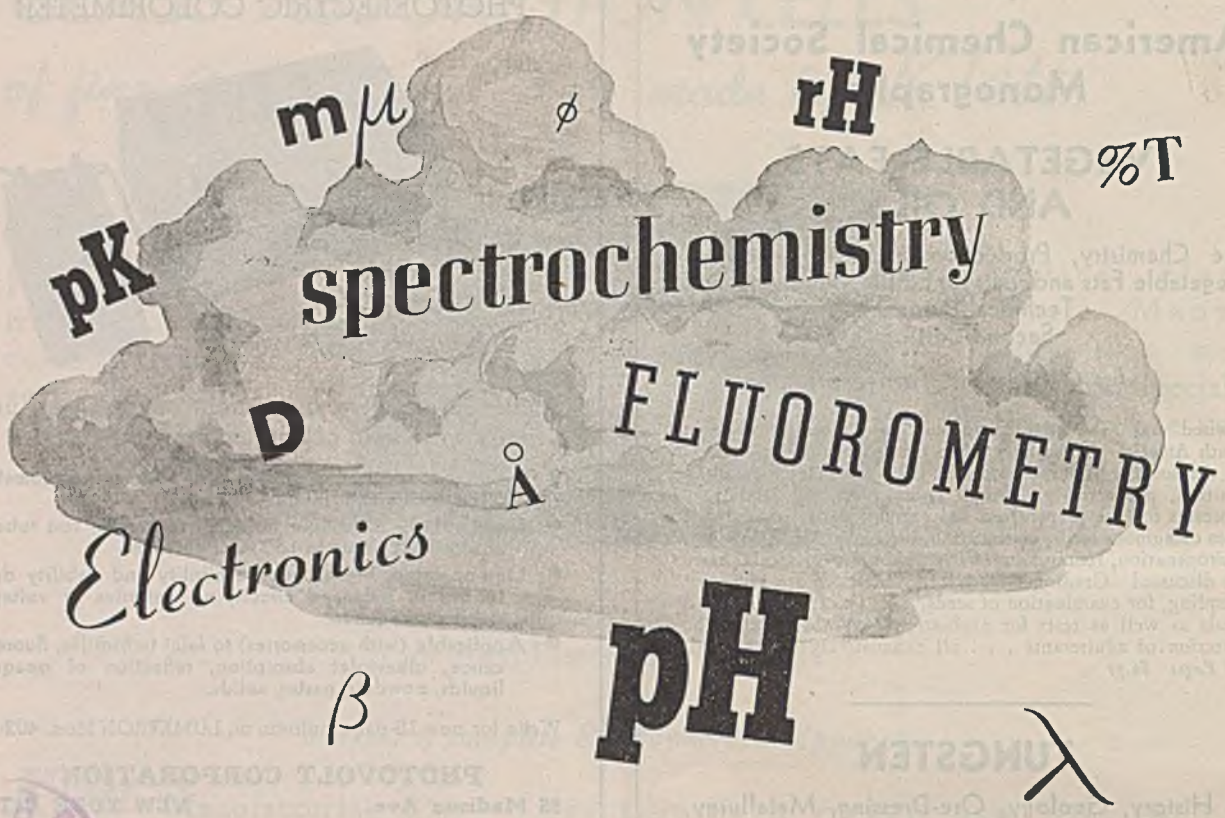

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